Guide to Laboratory Services: Microbiology

Arizona Department of Health Services Bureau of State Laboratory Services 250 North 17th Avenue Phoenix, Arizona 85007 (602) 542-1188

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General Information

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Director, Laboratory Services Daniel M. Lavine, M.D.

Assistant Bureau Chief William M. Slanta

Phoenix Laboratory

Hours of Operation: 8:00 AM to 5:00 PM Monday through Friday (Emergency services

available on nights or weekends when required by public health

needs.)

Annual Holiday Schedule: Laboratory Services observes all state recognized holidays.

Location: 250 North 17th Avenue, Phoenix, Arizona 85007

Telephone Number: (602) 542-1188

WATTS Line: (800) 525-8915

Fax Number: (602) 542-0760

Emergency Pager

(Weekends/After Hours): (602) 591-8683

Flagstaff Regional Laboratory

Hours of Operation: 8:00 AM to 5:00 PM Monday through Friday (Emergency services

available on nights or weekends when required by public health

needs.)

Annual Holiday Schedule: Laboratory Services observes all state recognized holidays.

Location: 2500 Fort Valley Road, PO Box 2800, Flagstaff, Arizona 86003

Telephone Number: (928) 226-1154

Fax Number: (928) 774-9419

Tucson Regional Laboratory

Hours of Operation: 8:00 AM to 5:00 PM Monday through Friday (Emergency services

available on nights or weekends when required by public health

needs.)

Annual Holiday Schedule: Laboratory Services observes all state recognized holidays.

Location: 416 West Congress Street, Tucson, Arizona 85701

Telephone Number: (520) 628-6360

Fax Number: (520) 628-6356

State Laboratory Contact Information

Section	Supervisor	Telephone Number
Receiving	Kathleen Rodriguez	(602) 542-1190
Special Pathogens: Bacteriology/Parasitology/Mycology/ Mycobacteriology Environmental Microbiology	Chris Mylott	(602) 542-6131
Newborn Screening Laboratory	Debbie Wright	(602) 542-1184
Serology	Mary Finnerty	(602) 542-6125
Virology	Ron Cheshier	(602) 542-6134
Flagstaff Regional Laboratory	Delores Tellez	(928) 226-1154
Tucson Regional Laboratory	Judith Fordyce	(520) 628-6360
BioEmergency Detection and Response (Bioterrorism)	Gary Cage	(602) 364-0999

Diagnostic Microbiology and Immunology Services

The policy of the Arizona State Laboratory is to provide routine clinical microbiology and immunology diagnostic support to county and state agencies. In addition, the State Laboratory serves as a reference microbiology laboratory to hospital and independent clinical laboratories in order to confirm their results from cultures and clinical specimens. This information is also used as part of the Department of Health Services disease surveillance program. Selected diagnostic test procedures are available to private medical practitioners when the procedure is not available on a statewide basis in private clinical laboratories and when intense surveillance is deemed necessary.

The State Laboratory provides specimen collection materials and mailers free of charge. Further information regarding specimen collection materials, mailing containers and *Request for Materials Form* is located in Section 11: Requesting Collection Kits and Mailing Containers. All requisitions and supplies for specimen submission are available through the Receiving Section, (602) 542-1190.

The purpose of this manual is to provide a ready reference to our clients and to assist them in obtaining laboratory services as efficiently as possible. The manual is distributed in loose-leaf format to allow for easy updating as procedures are modified or new testing is made available. Charts are provided for quick reference and more detailed information is available by test name in each section of the manual. Additional copies of this manual are available upon request from the State Laboratory and can be downloaded or viewed at www.azdhs.gov/lab.

Specimen Rejection Policy

The State Laboratory currently has the following policy for rejection of laboratory specimens and/or requested examinations. The State Laboratory will usually NOT examine clinical/reference specimens if the following circumstances exist:

- The quantity of specimen is not sufficient for examination.
- The specimen was too long in transit between the time of collection and receipt in the laboratory.
- The specimen was broken or leaked in transit.
- Clinical/epidemiological information submitted with the specimen was either insufficient or incomplete.
- Specimen was submitted in an improper container, transport media or preservative.
- Blood specimens are hemolyzed or contaminated.
- Only acute blood specimen was submitted, no convalescent specimen.
- The identifier on the specimen does not match the identifier on the submission form, or there is no identification on the specimen.
- Material for rabies examination is too decomposed to test.
- Test is available at a hospital/independent laboratory and has been discontinued by the State Laboratory.
- Reference cultures are contaminated.

Exceptions to this policy will be considered due to extenuating circumstances; however, final approval to make an exception will only be made by the Laboratory Director, Bureau Chief or Assistant Bureau Chief delegated this responsibility.

Directory of Laboratory Services

The following table lists the diagnostic and reference services offered by the Office of Public Health Microbiology. The table is organized alphabetically by disease or agent for easy referral. Please go to the specified laboratory section of this manual for more detailed information on collection and submission of laboratory samples.

		Antigen			Serology	Serology	Laboratory	Manual
Disease or Agent	Culture	Detection	Toxin	Smear	Single Serum	Paired Sera	Section	Location
Adenovirus	X					X	Virology	Section 6
	Λ					Λ	Serology	Section 5
Amoebiasis				X			Parasitology	Section 4
Anthrax	X						Bacteriology	Section 1
Arbovirus						X	Virology	Section 6
Albovilus						Λ	Serology	Section 5
Babesia				X			Parasitology	Section 4
Bacillus cereus	X						Bacteriology	Section 1
Datiuns terens	Λ						Environmental	Section 8
Blastomycosis	X						Mycology	Section 3
Botulism	X		X				CDC refer	
Brucellosis	X				X	X	Bacteriology	Section 1
Diucchosis	Λ				Λ	Λ	Serology	Section 5
Campylobacter	X						Bacteriology	Section 1
Candidiasis	X						Mycology	Section 3
							Bacteriology	Section 1
Chlamydia		X				X	Serology	Section 5
							Regular Labs	
Coccidioidomycosis	X				X		Mycology	Section 3
Coccidiolidomycosis	Λ				Λ		Serology	Section 5
Coxsackievirus	X					X	Virology	Section 6
Coasackievitus	Λ					Λ	Serology	Section 5
Cholera	X						Bacteriology	Section 1
	Λ						Environmental	Section 8
Colorado Tick Fever					X	X	Serology	Section 5

Disease or Agent	Culture	Antigen Detection	Toxin	Smear	Serology Single Serum	Serology Paired Sera	Laboratory Section	Manual Location
S		200000000000000000000000000000000000000	2 01111	0111001		1 011 00 0010	Virology	Section 6
CMV	X				X		Serology	Section 5
Cryptosporidium				X			Parasitology	Section 4
Cyclospora				X			Parasitology	Section 4
Dengue						X	Serology	Section 5
Diphtheria	X						Bacteriology	Section 1
E. coli	X						Bacteriology	Section 1
E. coll	A						Environmental	Section 8
Echovirus	X						Virology	Section 6
Ectoparasites							Parasitology	Section 4
Fascioliasis				X			Parasitology	Section 4
Filariasis				X			Parasitology	Section 4
Fungus	X						Mycology	Section 3
Giardiasis				X			Parasitology	Section 4
Gonorrhea	X	X					Bacteriology	Section 1
Gonormea	A	Λ					Regular Labs	
Hantavirus					X		Serology	Section 5
Haemophilus	X						Bacteriology	Section 1
Hepatitis					X		Serology	Section 5
Herpes	X					X	Virology	Section 6
-	Λ					Λ	Serology	Section 5
HIV					X		Serology	Section 5
Histoplasma	X				X	X	Mycology	Section 3
Пізторіазіна	A				Λ	Λ	Serology	Section 5
Influenza	X					X	Virology	Section 6
	21						Serology	Section 5
LCM						X	Serology	Section 5
Legionella	X						Bacteriology	Section 1
Leptospira	X						Bacteriology	Section 1
Listeria	X						Bacteriology	Section 1
LUSIVIU	21						Environmental	Section 8

		Antigen			Serology	Serology	Laboratory	Manual
Disease or Agent	Culture	Detection	Toxin	Smear	Single Serum	Paired Sera	Section	Location
Lyme					X		Serology	Section 5
Malaria				X			Parasitology	Section 4
Measles	X				X	X	Virology	Section 6
					Λ	Serology	Serology	Section 5
Meningococcus	X						Bacteriology	Section 1
Microsporidia				X			Parasitology	Section 4
Microfilaria				X			Parasitology	Section 4
Mites							Parasitology	Section 4
Mumps	X				X	X	Virology	Section 6
Wumps	Λ				Λ	Λ	Serology	Section 5
Murine Typhus						X	Serology	Section 5
Mycobacteria	X	HPLC					Mycobacteriology	Section 2
Mycoplasma						X	Serology	Section 5
Nocardia	X						Mycobacteriology	Section 2
Parainfluenza	X					X	Virology	Section 6
1 arammuchza	Λ					Λ	Serology	Section 5
Parasites				X			Parasitology	Section 4
Pertussis	X	X					Bacteriology	Section 1
Pinworm				X			Parasitology	Section 4
Plague	X						Bacteriology	Section 1
Pneumocystis				X			Parasitology	Section 4
Polio	X					X	Virology	Section 6
	Λ						Serology	Section 5
Psittacosis						X	Serology	Section 5
Q Fever					X	X	Serology	Section 5
Rabies				X			Virology	Section 6
RSV	X					X	Virology	Section 6
	Λ			Λ	Serology	Section 5		
Rocky Mountain Spotted Fever					X	X	Serology	Section 5

		Antigen			Serology	Serology	Laboratory	Manual
Disease or Agent	Culture	Detection	Toxin	Smear	Single Serum	Paired Sera	Section	Location
Rubella					X	X	Serology	Section 5
Salmonella	X						Bacteriology Environmental	Section 1 Section 8
SARS						X	Serology	
Schistosomiasis				X			Parasitology	Section 4
Shigella	X						Bacteriology	Section 1
St. Louis Encephalitis	X	PCR				Virology		
Staphylococcus	X						Bacteriology	Section 1
Streptococcus	X						Bacteriology	Section 1
Strongyloides				X			Parasitology	Section 4
Syphilis					X		Serology Regular Labs	Section 5
Toxoplasmosis					X		Serology	Section 5
Tuberculosis	X	HPLC/ PCR			X		Mycobacteriology Serology	Section 2
Tularemia	X						Bacteriology	Section 1
Varicella-Zoster	X				X	X	Virology Serology	Section 6 Section 5
West Nile	X	PCR			X		Virology Serology	
Western Equine Enceph	X	PCR					Virology	

Note HPLC = High Pressure Liquid Chromatography

PCR = Polymerase Chain Reaction

Section 1: Bacteriology

Upon receipt in the State Laboratory, all specimens are logged in and assigned to the appropriate area for processing. The time required to process a microbiology specimen varies considerably, as indicated by the following table. Detailed information on the collection and submission of laboratory samples on any of the following tests can be obtained in the following narrative guidelines.

During outbreaks, the Bureau of Epidemiology and Disease Control may conduct surveillance to determine the extent of the outbreak or to determine the relatedness of microorganisms identified in the outbreak. The Office of Microbiology will support these outbreak investigations through the use of various molecular tools, including plasmid electrophoresis and Pulsed Field Gel Electrophoresis (PFGE). Data may be shared in these investigations with other states and the CDC in the event of a multi-state outbreak.

Organism/ Disease	Specimen	Transport Medium	Comments	Turn Around Time (TAT)
Anthrax	Carcass, Hide, Hair, Bones, Vesicle, Blood	Standard transport medium or blood culture		5 days
Botulism	Serum, Feces, Food	None		Referred to CDC
Brucellosis	Blood, Bone Marrow, Biopsy	Blood Culture	Culture Specimens as soon as possible	7 days
Campylobacter	Feces	Cary-Blair	See Enteric Culture	3 days
Chlamydia PCR	Cervical, Urethral	Nucleic Acid Transport (NAT)		2 days
Cholera	Feces	Cary-Blair	Do not refrigerate	3 days
Diphtheria	Throat (membrane) or NP Swab	Pai or Loeffler slant		3 days
E. coli	Feces	Buffered Glycerol Saline or Cary-Blair		3 days
Enteric Culture	Feces	Buffered Glycerol Saline or Cary-Blair		3 days
Gonorrhea Culture	Cervical, Vaginal, Urethral	Thayer-Martin Plate	Transport by courier in a candle jar	3 days
Gonorrhea Culture- Reference Culture	Pure Culture	Thayer-Martin or chocolate agar		3 days
Gonorrhea PCR	Cervical, Vaginal, Urethral	Nucleic Acid Transport (NAT)		2 days
Haemophilus- Reference Culture	Pure Culture	Chocolate agar or Lewinthal agar slants		3 days

Organism/ Disease	Specimen	Transport Medium	Comments	Turn Around Time (TAT)
Legionella	Sputum or Lung Tissue	None		3 days
Leptospira	Blood, CSF, Urine	Blood: tubes containing heparin; CSF, Urine: None	Transport at 5° C- 20° C	6 weeks
Listeria	Blood, CSF	None	Ship at 4° C	5 days
Meningococcu s-Reference Culture	Pure Culture	Chocolate agar		3 days
Pertussis	Nasopharyngeal Swab	Reagan-Lowe	Use Dacron or calcium alginate swabs	5 days
Plague	Blood, Sputum, Aspirate, Biopsy, Necropsy	Cary-Blair		3 days
Salmonella- Reference Culture	Pure Culture	Culture Plate	See also Enteric Culture	3 days
Shigella- Reference Culture	Pure Culture	Culture Plate	See also Enteric Culture	3 days
Staphylococci- Reference Culture Only	Pure Culture	Culture Plate	Outbreak investigations only	5 days
Streptococci- Strep pneumo	Pure Culture	Culture Plate		Typed at State Lab
Tularemia	Skin lesions, Biopsy, Blood	Culture directly from tissues		5 days

Anthrax

Members of the genus are aerobic, gram-positive spore forming bacteria. *Bacillus anthracis* is the causative agent of anthrax.

Collection

In all cases, specimens from possible sources of infection (carcasses, hides, hair, bone) should be sought.

In cutaneous anthrax, swabs are appropriate for collection of vesicular exudate found in early lesions. When vesicular exudate is absent, fluid should be obtained by application of a capillary tube under the well-formed lesion.

If intestinal or pulmonary anthrax is suspected, blood should be cultured. Other specimens including hemorrhagic fluid from the mouth and nose, or anus should be collected in post-mortem cases. If they are negative, specimens of peritoneal fluid, spleen, and/or mesenteric lymph nodes may be collected.

Shipment of Specimens

Bacillus species are quite hardy and usually survive transport to the State Laboratory either in freshly collected specimens or in a standard transport medium.

See Section 10: Sample Submission Guidelines.

Reporting and Interpretation of Results

Cultures are incubated for 48 hours and checked daily for characteristic macroscopic morphology. Suspected isolates are tested biochemically. Confirmation is made by susceptibility to gamma phage. *B. anthracis* is inhibited and will not grow in the presence of gamma phage.

All results of positive cultures will be called to the submitting agencies, the appropriate county health department, and the Bureau of Epidemiology and Disease Control. Positive isolates of *B. anthracis* will be forwarded to the Centers for Disease Control in Atlanta, Georgia for confirmation of laboratory results.

Botulism

Collection

Infant Botulism

- 1. Serum for toxin 2.5 ml minimum
- 2. Stool for culture and toxin 20 to 50 grams (or as much as possible)
 - Toxin testing 10 to 30 grams
 - Culture 10 to 20 grams, or 15 to 25 ml of watery enema. In some cases, a rectal swab may be accepted, only if other stool specimens are not available.
- 3. Food for toxin and culture

Food borne Botulism - Adult*

- 1. Serum 15 to 20 ml
- 2. Feces -25 to 50 grams
- 3. Remainder of suspected food
- * Approval for adult botulism testing must be made from the Bureau of Epidemiology and Disease Control <u>prior to submission</u>. Contact the Infectious Disease Section of the Bureau of Epidemiology and Disease Control at (602) 364-3669.

Wound Botulism

- 1. Serum 15 to 20 ml
- 2. Feces -25 to 50 grams
- 3. Tissue, exudate or swab samples from wound

Shipment of Specimens

All specimens should be kept at refrigerated temperatures during storage and shipment. Shipment should contain ice or cool packs.

See Section 10: Sample Submission Guidelines.

All specimens will be forwarded on to the Centers for Disease Control in Atlanta, Georgia for testing.

Reporting and Interpretation of Results

The State Laboratory will notify by telephone the submitting agency and the Bureau of Epidemiology and Disease Control with results of the botulism testing as soon as they are available.

Brucellosis

Collection

Specimens that can be collected and cultured for the isolation of *Brucella* include blood*, bone marrow, biopsy, tissue aspirates, spleen and liver biopsies. Rarely, cerebrospinal fluid (CSF), pleural fluid, peritoneal fluid, and urine may be collected.

*When brucellosis is suspected, multiple blood cultures should be obtained.

Shipment of Specimens

Specimens should be cultured as soon as possible after collection or refrigerated if delays are unavoidable.

All specimens should be kept at refrigerated temperatures during shipment.

See Section 10: Sample Submission Guidelines.

Reporting and Interpretation of Results

Cultures for *Brucella* are held for 7 days and checked daily for typical growth. Suspected isolates are examined by Gram staining for typical microscopic morphology. Identification is made through biochemical testing. Confirmation of the isolate is made by testing for agglutination in monospecific sera and growth in the presence of dyes.

Chlamydia PCR

Collection

For reliable results, follow instructions below for proper specimen collection. This test is not intended for use with throat, rectal, or other specimen types than those indicated.

In order to insure proper delivery to the State Laboratory for testing, urine and urogenital swab specimens should be transported to the laboratory in as short a time as practical.

Cervical Swab Specimens

- 1. Remove excess mucous from the exocervix with one medium-cleaning swab provided in the (NAT) collection system and discard the swab.
- 2. Insert the second swab from the collection kit into the endocervix and rotate the swab for 15 30 seconds to ensure adequate sampling.
- 3. Withdraw the swab carefully and avoid any contact with the vaginal mucosa.
- 4. Holding the tube upright, verify that all of the Nucleic Acid Transportation (NAT) media is at the bottom of the transport tube. Unscrew the cap of the transport tube, fully insert the swab into the transport tube and break the swab at the score line. Screw the cap on securely.
- 5. Label the transport tube with the patient's ID and date of collection.

Urethral Swab Specimens

- 1. Make sure the patient has not urinated for at least one hour prior to sample collection.
- 2. Insert the small-tipped swab 2 to 4 cm into the urethra and rotate for 3 5 seconds to assure adequate sampling.
- 3. Withdraw the swab.
- 4. Holding the tube upright, verify that all of the NAT media is at the bottom of the transport tube. Unscrew the cap of the transport tube, fully insert the swab into the transport tube and break the swab at the score line. Screw the cap on securely.
- 5. Label the transport tube with the patient's ID and date of collection.

Urine (Male and Female)

Note: Patient must not have urinated during the previous two hours.

- 1. Collect 10 50 ml of the first catch urine (the first part of the stream) into a clean polypropylene container without preservatives.
- 2. Seal the specimen container and label appropriately. The specimen may be transported to the test site at room temperature (18°-30° C).

Shipment of Specimens

Swab Specimens

- 1. Swab specimens may be transported to the state laboratory in NAT media at 2° 28° C. If extremes in temperature are anticipated during transport, use of cool packs to maintain nucleic acid integrity is advised.
- 2. Swab specimens may be stored for up to 7 days from collection at 2° 28° C.
- 3. All specimens must be transported to the laboratory in compliance with state and federal regulations for transportation of etiologic agents. Temperature conditions must be maintained during transport. Use of cool packs in shipping is advised.
 - See Section 10: Sample Submission Guidelines.
- 4. Samples should be processed as soon as possible to avoid degradation of nucleic acids. If testing cannot be conducted within 7 days, samples may be frozen at -20° C for up to 30 days.

Urine Specimens

Urine specimens may be transported to the test site at 18° - 30° C. Urine specimens are stable for 24 hours at room temperature. Urine specimens that will not be processed within 24 hours of collection must be stored at 2° - 8° C and must be processed within 7 days of collection. Urine specimens that cannot be processed within 7 days of collection can be stored at -20° C or lower and tested within 30 days of collection.

Reporting and Interpretation of Results

The Amplicor CT/NG test for *Chlamydia trachomatis* is based on four main processing steps; specimen preparation, PCR amplification of target DNA using CT specific complementary primers, hybridization of the amplified DNA to the oligonucleotide probes, and detection of the probebound amplified DNA by colorimetric determination. Testing is performed daily in each of the state laboratories in Phoenix, Flagstaff and Tucson. Samples testing positive for GC are reported by phone to the submitting agency.

Cholera

Collection

Stool specimens should be collected early, preferably within 24 hours of onset of illness, and before administration of antibiotics. Rectal swabs or fecal material should be placed in the semisolid transport medium of Cary and Blair, which maintains the viability of vibrios for up to 4 weeks. Buffered glycerol-saline is an unsatisfactory transport medium, even for short periods.

Shipment of Specimens

Specimens in transport media should be shipped to the State Laboratory without refrigeration.

See Section 10: Sample Submission Guidelines.

Reporting and Interpretation of Results

Cultures suspected to contain *Vibrio cholera* are tested with commercial biochemical systems. Cultures presumptively identified as *Vibrio cholera* will be tested against specific antisera to determine the serogrouping of the isolate. *Vibrio cholera* strains will fall into two groups based on this serological testing. O group 1 strains (O1) are associated with epidemic cholera; non-O1 strains may cause cholera-like and other illnesses, but are not involved in epidemics. *Vibrio cholera* O1 strains are divided into three subtypes: Ogawa, Inaba, and Hikojima. The O1 strains are further divided into two biogroups: classical and El Tor.

Diphtheria

Collection

Both throat swabs and nasopharyngeal swabs should be collected from patients suspected of having Diphtheria.

The swabs should be placed on Pai or Loeffler slants and transported to the State Laboratory. Alternately, swabs can be transported in a silica gel packet if no other media is available. However, there may be significant loss of *C. diphtheriae* if this method is used.

Shipment of Specimens

The State Laboratory should be notified <u>24 hours in advance</u> of a specimen submission. The specimen should be immediately transported to the State Laboratory or inoculated onto proper media. Transport on Pai or Loeffler slants or in a silica gel packet is appropriate.

See Section 10: Sample Submission Guidelines.

Reporting and Interpretation of Results

Cultures will be examined for 48 hours and observed daily for typical growth characteristics. Suspicious colonies are checked microscopically for typical morphology, and identification of suspected isolates will be made using biochemical tests. Positive cultures of *C. diphtheriae* are classified into four biotypes based upon their colonial morphology and biochemical reactions. Negative cultures will be held for at least 48 hours before reporting as negative.

Cultures identified positive for *C. diphtheriae* will be tested for virulence production using in vitro toxigenicity studies.

Positive reports of *C. diphtheriae* will be telephoned to the submitting agency, the County Health Department, and the Bureau of Epidemiology and Disease Control.

E. coli 0157

See Enteric Culture Page 1-10

Enteric Cultures

Collection

The most often cultured sources for enteric diseases are feces, blood, and urine. Other extraintestinal sources may be infected with enteric disease organisms. Purulent material from wounds or abscesses may be swabbed or aspirated for the presence of *Salmonella sp.* Sediment from spinal fluid, sputum, nasopharyngeal swabs, exudates, and other sources may be successfully cultured.

- Stool specimens should be taken early in the course of illness when the causative agent is likely to be present in the largest numbers. Freshly passed stool is better than rectal swabs since there is less chance of improper collection, and mucus and bloodstained portions can be selected for culture. Collect a small portion of feces, approximately the size of a marble, or a swab coated with feces and place in a transport medium. Whenever possible, multiple specimens should be cultured. The State Laboratory will provide agencies with both a Cary-Blair transport medium and buffered glycerol-saline. It is important to inoculate the specimen to both transport media. Cary-Blair is the best overall transport medium for diarrheal stools.
- Blood for culture should be collected during those times when a patient's temperature is rising or falling since the maximum number of positive cultures may not be obtained from samples drawn at the peak of a temperature curve. Multiple specimens should be collected, as many as five or six, drawn at 1-2 hour intervals.
- Midstream urine samples should be examined as soon as possible after collection, since it is known that misleading results may be obtained if bacteria are allowed to proliferate during the time from collection of the specimen until the time it is cultured.
- Reference isolates of *Salmonella* and *Shigella* for epidemiological studies. Transfer isolate to a TSI or nutrient agar slant and forward to the State Laboratory in Phoenix.

Shipment of Specimens

Specimens held in transport media should be refrigerated until examined. Transport specimens to the State Laboratory in Phoenix at a refrigerated temperature in the proper transport media. See Section 10: Sample Submission Guidelines.

Reporting and Interpretation of Results

Stool samples, unless otherwise specified, will be screened for *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia* (upon request), and *E. coli* O157. Cultures are examined daily for 48 hours for characteristic morphology. Suspect colonies are screened biochemically, and confirmed with serologic agglutination (where applicable). Organisms in the genus *Salmonella* are typed using both somatic and flagellar antisera.

All reports of Salmonella typhi and Shigella dysenteria are telephoned to the submitting agency and to the Bureau of Epidemiology and Disease Control.

Gonorrhea Culture

Diagnostic GC Culture

Collection

Specimens may be collected from a number of anatomical sites. In screening clinic programs, the most common sites are the cervix for females and the urethra for males. The following are collection recommendations from "A Guide for the Diagnosis of Gonorrhea", USDHHS-PHS, CDC.

Endocervical Canal

- 1. Lubricate any instruments with warm water only, as other lubricants may be toxic for gonococci.
- 2. After inserting speculum, remove excess mucus with a cotton ball.
- 3. Insert sterile cotton tipped swab into endocervical canal, move side to side, allow 10 20 seconds for adsorption of organisms onto the swab.

Vaginal

1. Use cotton swab to obtain specimen from the posterior vaginal vault or obtain specimen from the vaginal orifice if the hymen is intact.

Urethral

- 1. Collect urethral specimens at least one hour after the patient has urinated.
- 2. Purulent discharge can be collected directly on a swab.
- 3. If no discharge, insert a sterile urethral swab 2-cm into the urethra and rotate the swab gently as it is withdrawn.

Anal - Rectal

- 1. Insert a cotton-tipped swab 4 to 5 cm into the anal canal.
- 2. Move the swab from side to side to sample the crypts.
- 3. If fecal contamination occurs, discard swab and use another to obtain the specimen.

Throat

1. Swab the posterior pharynx and tonsillar crypts with a cotton-tipped applicator.

Other Sites

- 1. Conjunctiva
- 2. Joint Fluid
- 3. Prostatic Fluid
- 4. Cutaneous Lesions

All specimens must be inoculated onto plates of Modified Thayer Martin (MTM) agar, labeled on the back with patient name, date of birth, and site using the following pattern:

- 1. Roll the swab across the surface of the media in a large Z pattern.
- 2. Immediately cross the streak with the tip of the swab.
- 3. Using a small piece of Scotch tape, secure the lid to the plate.
- 4. Put the inoculated plate into a candle jar, media-side up.
- 5. Incubate the plates for 24 hours at $35^{\circ} \pm 2^{\circ}$ C.

Shipment of Specimens

Once the MTM plates have been inoculated and placed incubated for 24 hours in a candle jar, the specimens should be transported by courier from the clinic to the State Laboratory in the shortest time possible to maintain the viability of the specimens.

See Section 10: Sample Submission Guidelines.

If the specimens are to be held at the clinic, the candle jars must be incubated between 33° and 37°C no more than 3 days before transport to the State Laboratory.

Reporting and Interpretation of Results

Cultures are read daily for 3 days before reporting out a negative culture. Oxidase tests are performed on all suspicious colonies. Confirmatory tests are performed on all isolates from urogenital sites and for all isolates from other body sites.

Results of positive GC cultures are reported by telephone to the submitting agency.

Reference GC Culture

Reference specimens submitted for confirmation of identification of *Neisseria gonorrhea* may be submitted to the State Laboratory in Phoenix. Culture plates or chocolate agar slants containing the bacterium should be mailed or transported to the laboratory as quickly as possible. Gonococci autolyze as they age, and the culture becomes nonviable.

Reporting and Interpretation of Results

A fresh subculture of the organism is required for the purposes of performing biochemical tests. Organisms presumptively identified as *Neisseria gonorrhea* require confirmatory testing. The cultures are confirmed using Direct Fluorescent Antibody (DFA) reagents.

Gonorrhea PCR

Collection

To obtain reliable results, follow the instructions below regarding appropriate specimen collection. This test is not intended for use with throat, rectal, or specimen types other than those indicated.

In order to ensure proper delivery to the laboratory for testing, urine and urogenital swab specimens should be transported to the laboratory in as short a time as practical. Do not allow specimens to be transported without controlled temperature conditions.

Cervical swab specimens

- 1. Remove excess mucous from the exocervix with one medium-cleaning swab provided in the collection system (NAT) and discard the swab.
- 2. Insert the second swab from the collection kit into the endocervix and rotate the swab for 15 30 seconds to ensure adequate sampling.
- 3. Withdraw the swab carefully and avoid any contact with the vaginal mucosa.
- 4. Holding the tube upright, verify that all of the NAT media is at the bottom of the transport tube. Unscrew the cap of the transport tube, fully insert the swab into the transport tube and break the swab at the score line. Screw the cap on securely.
- 5. Label the transport tube with the patient's ID and date of collection.

Urethral swab specimens

- 1. Make sure that the patient has not urinated for at least two hours prior to sample collection.
- 2. Insert the small-tipped swab 2 to 4 cm into the urethra and rotate for 3 5 seconds to assure adequate sampling.
- 3. Withdraw the swab.
- 4. Holding the tube upright, verify that all of the NAT media is at the bottom of the transport tube. Unscrew the cap of the transport tube, fully insert the swab into the transport tube and break the swab at the score line. Screw the cap on securely.
- 5. Label the transport tube with the patient's ID and date of collection.

Male Urine (This procedure has not been approved for female urines.) Note: Patient must not have urinated during the previous two hours.

- 1. Collect 10 50 ml of the first catch urine (the first part of the stream) into a clean polypropylene container without preservatives.
- 2. Seal the specimen container and label appropriately. The specimen may be transported to the test site at room temperature (18°-30° C).

Shipment of Specimens

Swab Specimens

- 1. Swab specimens may be transferred to the laboratory at 2° 28° C. If extremes in temperature are anticipated during transport, use of cool packs to maintain nucleic acid integrity is advised.
- 2. Swab specimens may be stored for up to 7 days from collection at 2° 28° C.
- 3. All specimens must be transported to the laboratory in compliance with state and federal regulations for transportation of etiologic agents. Temperature conditions must be maintained during transport. Use of cool packs in shipping is advised.
- 4. Samples should be processed as soon as possible to avoid degradation of nucleic acids. If testing cannot be conducted within 7 days, samples may be frozen at -20° C for up to 30 days.

Urine Specimens

Urine specimens may be transported to the test site at 18° - 30° C. Urine specimens are stable for 24 hours at room temperature. Urine specimens that will not be processed within 24 hours of collection must be stored at 2° - 8° C and must be processed within 7 days of collection. Urine specimens that cannot be processed within 7 days of collection can be stored at -20° C or lower and tested within 30 days of collection.

See Section 10: Sample Submission Guidelines.

Note: Submit specimens for Gonorrhea PCR to Flagstaff or Tucson Regional Labs.

Reporting and Interpretation of Results

The Amplicor CT/NG test for *Neisseria gonorrhea* is based on four main processing steps; specimen preparation, PCR amplification of target DNA using GC specific complementary primers, hybridization of the amplified DNA to the oligonucleotide probes, and detection of the probebound amplified DNA by colorimetric determination. Testing is performed daily in each of the Flagstaff and Tucson laboratories. Samples testing positive for GC are reported by telephone to the submitting agency.

Haemophilus

Collection

H. influenzae

Specimens must be collected and cultured as soon as possible since the organisms do not survive well. Pure culture isolates from sterile sites such as blood or cerebrospinal fluid may be submitted to the State Laboratory. It is not recommended that clinical materials be submitted to reference laboratories for isolation.

Note: Isolates should be transported on chocolate slants.

H. aegyptius

This organism is closely related to *H. influenza*, and is the causative agent of contagious conjunctivitis. Conjunctival scrapings should be collected and cultured immediately. Pus may be collected on the tip of a calcium alginate swab and placed in a modified Stuarts Transport medium prior to culture. Reference isolates may be forwarded on to the State Laboratory for confirmation.

H. ducreyi

Chancroid lesions should be carefully scraped or swabbed. These specimens should not be allowed to dry, and should be cultured immediately.

Shipment of Specimens

Reference isolates should be transported on slants of chocolate agar or Lewinthal agar. Both *H. aegyptius* and *H. ducreyi*, because of their fastidious nature, should be transported on chocolate agar slants supplemented with 1% IsoVitalex.

See Section 10: Sample Submission Guidelines.

Reporting and Interpretation of Results

H. influenza serotype b has been identified as the leading cause of bacterial meningitis and epiglottitis. It has also been implicated as a major cause of pericarditis, pneumonia, septic arthritis, osteomyelitis, and facial cellulitis, as well as an occasional cause of urinary tract infection in children less than 5 years of age. Non-encapsulated strains noninvasive respiratory infections in healthy children, community acquired pneumonia and chronic bronchitis in adults.

Biotyping of *H. influenza*, *H. parainfluenza*, as well as the identification of *H. segnis* and the aegyptius biogroup of *H. influenza* can be accomplished with the biochemical tests provided at the State Laboratory.

Serotyping is relevant only for the encapsulated strains of *H. influenza*. Testing is performed using rapid agglutination techniques in type-specific antisera.

Legionella

Collection

Legionellae are most frequently isolated from specimens originating in the respiratory tract. On rare occasions, they may be isolated from extra-pulmonary sites including pericardial fluid, peritoneal fluid, wounds, and abscesses. Legionellae are not known to colonize humans, and therefore are not commensals of the respiratory tract. Respiratory secretions from those patients who are not able to provide adequate sputum specimens may be collected by transtracheal aspiration or bronchoalveolar lavage. On occasion, it may be necessary to collect lung tissue samples to establish the diagnosis of Legionnaires Disease.

Sputum should be collected and transported in sterile containers with tight fitting lids. Use of saline in specimen collection fluids should be avoided, since sodium ions may be inhibitory to the organism.

Shipment of Specimens

Special media are not required for transport of specimens, as long as they are protected from drying and rapid temperature changes. Specimens can be held at 4° C or transported on wet ice, provided they are examined within 48 hours of collection. Those that are to be held for longer periods should be stored frozen, preferably at -70° C and transported in the frozen state.

See Section 10: Sample Submission Guidelines.

Reporting and Interpretation of Results

Cultures are incubated and examined daily for the presence of *Legionella*. Organisms are presumptively identified as *Legionella* by demonstrating the isolate is a gram-negative rod that requires L-cysteine for growth. The organism is then serogrouped using an IFA test with commercially available FITC-conjugated antisera.

Leptospira

Collection

Blood, cerebrospinal fluid (CSF), and urine are the specimens of choice for recovery of leptospires. The most appropriate choices to culture during the first 10 days of illness are blood and CSF. The specimens should be collected prior to antibiotic treatment and while the patient is febrile. After the first week of illness, the optimal source for isolation of leptospires is urine.

If culture medium is not available, blood should be collected in tubes containing heparin or sodium oxalate. Tubes containing citrate should be avoided, since citrate may be inhibitory.

Shipment of Specimens

Blood and CSF specimens should be stored and transported at 5° - 20° C and inoculated into culture medium within one week of collection.

Urine should be inoculated as soon as possible, especially if the urine is acidic. If culture media is not immediately available, the urine can be diluted 1:10 in 1% bovine serum albumin and stored for a few days at 5° - 20° C.

See Section 10: Sample Submission Guidelines.

Reporting and Interpretation of Results

All leptospiral cultures are held at room temperature (5° - 20° C). Cultures are examined weekly by darkfield microscope examination for the presence of leptospires. Cultures are held for 6 weeks before reporting a culture negative. All isolates of *Leptospira* will be forwarded to the CDC for confirmation.

Listeria

Collection

Clinical specimens from normally sterile sites such as blood, cerebrospinal fluid (CSF) amniotic fluid, placenta, or fetal tissue do not require special procedures for collection or transport. Specimens from non-sterile sites, such as meconium, feces, vaginal secretions, respiratory, skin or mucous swabs require prompt handling to prevent the overgrowth of contaminants.

Culture specimens from sterile sites can be plated directly to tryptic soy agar containing 5% sheep, horse, or rabbit blood. Samples for blood culture can be inoculated directly into conventional blood culture broth.

Shipment of Specimens

Specimens from sterile sites should be transported as soon as possible. If processing is delayed, specimens should be held at 35° C in an incubator for no longer than 48 hours. Specimens from non-sterile sites require prompt handling. If processing is delayed, the materials should be kept at 4°C or frozen at -20° C if testing delays are expected to exceed 48 hours. Ship at 4° C.

Non-sterile specimens (other than stool) can be stored at 4° C for up to 48 hours. For longer periods of storage, freezing specimens at -20° C is recommended.

Stools should be shipped frozen on dry ice.

Reference cultures can be transported on Nutrient Agar slants or other non-glucose containing agar slants at ambient temperature.

See Section 10: Sample Submission Guidelines.

Reporting and Interpretation of Results

Inoculated media will be incubated for 5 to 7 days and examined daily for growth. Isolates and reference specimens are streaked to a blood agar plate and examined daily for typical growth characteristics. Identification of *Listeria* is made based on colonial morphology (including beta hemolysis, Gram-stain, catalase, oxidase and motility), microscopic morphology, and various biochemical reactions.

Meningococcus

Collection

Specimens from which *Neisseria meningitidis* may be isolated include CSF, blood, petechial aspirates, biopsy samples, joint fluid, and conjunctival swabs.

Inoculate specimens directly onto a nutritive, nonselective medium such as chocolate medium supplemented with IsoVitaleX or a blood agar medium and incubate in a CO₂ enriched atmosphere immediately after collection.

Shipment of Specimens

Transport specimens or reference isolates as quickly as possible to the State Laboratory. It is recommended that the containers be insulated during very hot or very cold weather. All cultures must be transported with minimum delay since viability is readily lost. If specimens must be transported to a distant town, the inoculated media must be incubated 18 - 24 hours before transport, and the specimen should arrive within 48 hours.

See Section 10: Sample Submission Guidelines.

Reporting and Interpretation of Results

Cultures are examined daily for typical growth characteristics. Isolates are identified biochemically. *N. meningitidis* isolates are serotyped for epidemiological purposes using type-specific antisera. During meningococcal outbreaks, molecular typing of isolates using Pulsed Field Gel Electrophoresis is used to aid in the outbreak investigation.

Pertussis

Collection

The specimen of choice for the recovery of *Bordetella pertussis* and *B. parapertussis* from the respiratory tract is secretions collected from the posterior nasopharynx. Specimens collected from the throat are not recommended. NP specimens may be collected as aspirates obtained by suction or perinasal swab specimens.

One or two perinasal swab specimens are collected by passing the swabs through the nares as far as possible into the nasopharynx. Leave the swab in place for up to 30 seconds. If resistance is encountered during insertion, try the other nostril. Rotate the swabs for a few seconds, and gently withdraw them.

Use only Dacron or calcium alginate swabs. *Bordetella pertussis* is killed by the fatty acids found in cotton swabs.

Push the swab, post collection, into a tube of Regan-Lowe semi-solid transport agar. Leave the swab submerged during transport to the laboratory.

Shipment of Specimens

If possible, the cultures should be transported on ice. If transport to the laboratory is delayed, specimens should be refrigerated. Transport to the laboratory either by courier or through the mail.

Reference isolates of B. Pertussis may be submitted to the State Laboratory for confirmation.

See Section 10: Sample Submission Guidelines.

Reporting and Interpretation of Results

A direct fluorescent antibody test is not routinely performed, but may be offered under a special request. Identification is made based upon colonial morphology, microscopic appearance, and biochemical testing. Cultures are confirmed by a Fluorescent Antibody test.

Results of positive cultures are telephoned to the submitting agency, the appropriate county health department, and to the Immunization Section of the Bureau of Epidemiology and Disease Control.

Plague

Collection

Clinical samples that may be submitted to the laboratory for identification of *Yersinia pestis* include blood, sputum, aspirates, biopsy and necropsy materials.

Animals and parasites may also be sent for isolation identification procedures.

Shipment of Specimens

Transport samples to the State Laboratory in Phoenix. Fleas and ticks will be forwarded on to the Plague Branch, Division of Vector-Borne Infectious Diseases at Fort Collins, Colorado.

Bubos or lymph nodes (tissues) should be collected into a broth medium to initiate growth. Cary and Blair transport may also be used. Whenever a clinical sample is taken for Plague culturing, always include serum samples (acute and convalescent).

If animals are collected, the entire animal should be submitted in a plastic bag with dry ice. If organs are removed, be sure to include spleen and liver. Send tissue samples frozen, and on dry ice.

If external parasites are collected, submit specimens in the following manner:

Fleas

Preserve in vials containing a mixture of 2% saline and 0.1% tween 80. Do not freeze or place in alcohol.

Ticks

Place in dry vials with no preservative. Do not freeze or place in alcohol.

See Section 10: Sample Submission Guidelines.

Reporting and Interpretation of Results

Cultures are identified by observing typical colonial morphology. Typical colonies are presumptively identified by use of a Direct Fluorescent Antibody (DFA) test. A positive DFA test is a presumptive positive for Plague. All DFA positive results are telephoned to the submitting agency and to the Vector Borne and Zoonotic Disease Section of Epidemiology and Disease Control. Cultures suspected of containing Plague are tested biochemically using conventional biochemicals. All cultures which test presumptively positive by DFA and are biochemically identified to be *Yersinia pestis* are confirmed as positive by the use of Phage strips. *Y. pestis* impregnated bacteriophage strips are used to differentiate unknown cultures from *Y. pseudotuberculosis*.

Negative cultures are held for 48 hours before reporting as negative.

Salmonella

See Enteric Culture Page 1-10

Shigella

See Enteric Culture Page 1-10

Tularemia

Collection

During infection, direct isolation is achieved from ulcer scrapings, lymph node biopsies, gastric washings, sputum, pharyngeal washes, and pleural fluid. Circulating blood seldom reveals the organism. In human cases, several sources should be considered. Organisms are invariably present in significant numbers in fluid from obvious local lesions. Skin around the lesion should be cleansed with alcohol and allowed to dry before opening the papule and exposing the fluid. Organisms may persist for long periods of time in lymph nodes and may be isolated by node biopsy.

Note: Specimens suspected of containing *Francisella tularensis* should be collected and submitted with extreme caution. Tularemia is currently listed as the third most common reported laboratory-associated bacterial infection.

Shipment of Specimens

Transport samples to the State Laboratory in Phoenix. Fleas and ticks will be forwarded on to the Plague Branch, Division of Vector-Borne Infectious Diseases at Fort Collins, Colorado.

If animals are collected, the entire animal should be submitted in a plastic bag with dry ice. If organs are removed, be sure to include spleen and liver. Send tissue samples frozen, and on dry ice. If external parasites are collected, submit the specimens in the following manner:

Fleas

Preserve in vials containing a mixture of 2% saline and 0.1% tween 80. Do not freeze or place in alcohol.

Ticks

Place in dry vials with no preservative. Do not freeze or place in alcohol.

See Section 10: Sample Submission Guidelines.

Reporting and Interpretation of Results

F. tularensis requires an enriched medium for growth. The historic medium of choice is cystine glucose blood agar. Cultures are plated onto both cystine heart agar and charcoal yeast extract with blood. Cultures are observed for 72 hours before reporting as negative. Cultures are observed for typical colonial morphology. Suspect colonies are checked microscopically by Gram staining, where they appear as faintly staining gram-negative coccobacilli. Confirmation of the isolate is determined by Direct Fluorescent Antibody and serological agglutination testing.

All positive cultures are reported to the submitting agency and the Vector Borne and Zoonotic Disease Section of the Bureau of Epidemiology and Disease Control. All *F. tularensis* isolates are forwarded to the Plague Branch of the Centers for Disease Control at Fort Collins, Colorado.

Section 2: Mycobacteriology

The State Laboratory provides diagnostic and reference services for the isolation and identification of *Mycobacterium tuberculosis* (MTB) and other mycobacteria at no charge to all public and private health care providers in the state. The State Laboratory receives a federal grant to support the statewide testing of *Mycobacterium* in support of the National Action Plan for the Elimination of Tuberculosis in the United States.

Collection

Use a clean, sterile, leak-proof disposable screw-capped 50 ml conical centrifuge tube supplied by the State Laboratory. <u>Do not use waxed containers</u>. More detailed information regarding how to obtain specimen collection and submitting materials can be found Section 10: Sample Submission Guidelines and Section 11: Requesting Collection Kits and Mailing Containers.

Sputum

In pulmonary tuberculosis and the related Mycobacterial diseases, sputum is the specimen of choice. A 5 - 10 ml sample of sputum is the desired volume for a single examination. Pooled specimens collected over several hours are not suitable for examination. A series of 3 early morning specimens, collected on consecutive days should be obtained. Collect the initial specimens before antimicrobial therapy is started. Do not use fixatives or preservatives.

Urine

The specimen of choice is a clean catch, midstream, first morning specimen. Urine should be collected in a clean, sterile, screw-capped plastic container. Pooled specimens or 24-hour urines are unacceptable. A series of first morning specimens should be collected on three consecutive days.

Gastric Washings

Gastric washings are specimens of last resort because they are highly diluted with gastric fluid, which is damaging to the tubercle bacillus. Specimens should be delivered to the laboratory immediately so neutralization procedures can begin. These samples are not suitable for mailing.

Specimens from Sterile Sites

These include cerebrospinal fluid (CSF), pleural fluid, ascitic fluid, joint fluid, pus, exudates, biopsy, and autopsy tissues. These are all surgical specimens and should be collected or taken by a physician or surgeon and placed in sterile containers. Tissue may be delivered in sterile saline. Do not add any preservatives.

Shipment of Specimens

After collection, identify the specimen with the patient's name and collection date. Fill out the proper laboratory submission form, *Microbiology Submission Form #2* (included with the specimen container obtained from the State Laboratory). Include the patient's name, date of birth, submitting agency, test request, and other pertinent demographic information.

Specimens should be refrigerated immediately after collection, prior to shipment. If specimens are to be shipped, it is necessary to place the specimen tube in a double mailing container to avoid contamination in the event of leakage. The desired mailing container consists of an inner metal screw-capped container placed within a screw-capped cardboard outer mailer. These containers are provided by the State Laboratory upon request. Place the submission form around the outside of the inner metal container. Never place the form inside this inner metal liner. The double mailer is a safety requirement and a postal shipping mandate. Mail as soon as possible after collection to avoid overgrowth of contaminating bacteria.

Reference specimens may be submitted in tubed solid media or in a liquid culture medium, including Bactec, MGIT, MB-Bacti, and Septi-Chek. Reference specimens that are mailed or delivered by courier transport must be placed in a double mailing container. In the event of courier transportation, the specimen may be sent in a 50 ml conical centrifuge tube inside an inner metal container and then placed in a sealed plastic bag. Securely tighten all caps.

See Section 10: Sample Submission Guidelines.

Reporting and Interpretation of Results

Specimens are processed daily, seven days a week. Smears are examined daily by fluorescent microscopy, using a fluorochrome stain. The results of positive smears are telephoned to the submitting agency within 24 hours. Preliminary laboratory reports are prepared and sent out for all smear results.

Specimens are cultured onto both solid and liquid media. Cultures are examined for growth during a period of 6 weeks (on negative smears) and 8 weeks for blood culture, before being reported as "No Growth". Cultures exhibiting typical colonial morphology are identified using High Performance Liquid Chromatography (HPLC). HPLC can be performed on cultures from both liquid and solid media. Allow 48 hours after detection of growth for identification of the organism. This method can be used to identify all known species of Mycobacteria.

Note: Rapid HPLC is being performed for the preliminary identification of *Mycobacterium sp.* from the pellets of specimen concentrates that have been determined to be smear positive. This presumptive identification can be reported within 24 hours of receiving the specimen in the State Laboratory. Confirmation of these findings is performed using the HPLC on the culture isolate and also direct PCR for the MTB complex.

Drug Susceptibilities

Direct drug susceptibility testing is performed on all newly identified patients with smear positive specimens. Results of the direct susceptibilities are available within 3 weeks. Indirect drug susceptibilities are performed only on *Mycobacterium tuberculosis* (MTB). This is routinely performed by the Bactec method. If the MTB is resistant to any of the drugs tested by Bactec, an indirect susceptibility is performed by the conventional proportional count method, where an additional drug regimen is tested. Drugs tested by the Bactec method are INH, PZA, Rifampin, Streptomycin, and Ethambutol. The proportional count method includes the same drugs tested by Bactec plus Ethionamide, Kanamycin, and Capreomycin. Susceptibilities are performed every 3 months on specimens that remain positive for MTB. All susceptibility results are telephoned to the submitter.

The results of all specimens are reported by mail to the submitter. In addition, all positive results are reported to the Tuberculosis Elimination Section of the Bureau of Epidemiology and Disease Control, Arizona Department of Health Services.

Section 3: Mycology

In order to successfully isolate pathogenic fungi from clinical material, specimens must be properly collected and as fresh as possible. Specimens from non-sterile sites, such as sputum and bronchial washings, may become overwhelmed with contaminants. Whenever possible, clinical materials for the isolation of mycotic agents should be handled by a local laboratory and isolates referred to the State Laboratory for identification and confirmation.

Collection

- Sputum specimens should be collected as a first morning specimen not exceeding 10 ml in volume. Collect in a clean, sterile, wide mouthed glass or plastic container with a screw-capped lid. DO NOT COLLECT POOLED SPECIMENS.
- Specimens from normally sterile sources (CSF, body fluids, and tissues) should be collected aseptically and placed in sterile containers. Large amounts of body fluids are preferred (a minimum of 5 ml of CSF should be collected). Tissues may be placed in a small amount of sterile saline to prevent drying.
- If Histoplasmosis or Coccidioidomycosis is suspected, bone marrow and peripheral blood may be submitted. Blood should be collected in a sterile vacutainer tube (approximately 10 ml) containing heparin as an anticoagulant. Bone marrow should be collected in a heparinized syringe and then placed in a sterile, screw cap container.
- Specimens for examination of dermatophytes (skin, hair, nails) should be collected and placed in a sterile container. Do not use any preservatives.

Shipment of Specimens

Specimens should be transported in sterile, leak-proof containers. Specimen containers must be enclosed in a secondary container to avoid leakage and contamination during transit. Reference cultures in tubes should be sent in double mailing containers. Tubes should be wrapped carefully to protect against breakage. Cultures should not be submitted on plated media due to potential safety hazards.

If delays in transit are expected, dermatological specimens should be stored at room temperature, and all other specimens at 4° C. Specimens submitted on Culturette swabs should not be stored before culturing, since *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Cryptococcus neoformans* may be inhibited.

The specimen must be identified with the patient's name and collection date. Specimens that are not properly identified will be rejected.

Each specimen must be accompanied by laboratory submitting form, *Microbiology Submission Form #2*. This submission form must be completed with the patient's name, date of collection, specimen source, test requested, and submitting agency, along with any additional pertinent demographic information.

See Section 10: Sample Submission Guidelines.

Reporting and Interpretation of Results

Fungal cultures are examined for a period of 4 weeks before being reported as negative. Positive cultures are identified by widely varied techniques. Yeast isolates are tested biochemically to determine their ability to assimilate and ferment various sugars. Molds are examined macroscopically and microscopically. Growth characteristics, such as ability to grow at different temperatures, growth in the presence of mycobiotics, and the organisms ability to perforate hair may all be taken into account. Histoplasma, Coccidioides, and Blastomyces are confirmed by an exoantigen test (Genprobe).

Section 4: Parasitology

Intestinal and blood parasites are diagnosed mainly by morphologic examination of diagnostic stages of the microorganism. Properly collected and preserved specimens are of the utmost importance, since old or poorly preserved materials are of little value in establishing a diagnosis and may lead to erroneous conclusions.

Collection

Fecal specimens

Collect the stool in a clean container or on clean paper and transfer to the Ova and Parasite transport containers supplied by the State Laboratory. The collection kit provided includes a container with PVA fixative and one container with 10% formalin fixative. A portion of the specimen, approximately 1 tablespoon, is added to the fixative in a ratio of 1 part specimen to 3 parts fixative. Mix thoroughly to assure adequate fixation. Do not contaminate specimen with urine or dirt. Administration of barium, magnesia, or oil before collection will render the specimen unsuitable for testing. Do not fill the vials more than half full. Label each vial with patient's name and address. Because the host passes parasites intermittently, multiple specimens should be examined. These irregularities emphasize the need to collect at least three specimens over 10 to 14 days.

Pinworm

The eggs of *Enterobius vermicularis* are usually collected with anal swabs or cellulose tape slides. Specimens taken between 10 PM and midnight or early morning before defecation are best. Three consecutive examinations are desirable. Specimens should be refrigerated if examination must be delayed for more than one day.

Worms

Whole worms or proglottids should be preserved in 70% alcohol in a screw capped plastic or glass container.

Blood Parasites

Blood films are best made from blood not containing anticoagulants, since anticoagulants can interfere with parasite morphology and staining. For routine diagnosis, a thick film is preferable, however parasite morphology is more distinct and typical when observed in a thin film. Therefore, it is important to collect both thick and thin films for submission. Thin films are made by depositing a single drop of blood at one end of the slide and spreading it across the slide in preparation for a differential count. Thick films are prepared by touching the under-surface of a slide with a fresh drop of blood from a finger (without touching the skin) and rotating the slide to form a film about the size of a dime. Alternately, several drops of blood can be deposited at the end of a slide and puddle with an applicator stick or toothpick. Allow 8 - 12 hours drying time for a thick film. Specimen slides should be placed in a cardboard slide holder, and labeled with proper identification.

If necessary, thick and thin films can be prepared from anticoagulated blood, but the staining characteristics are not as good. EDTA anticoagulated blood is better for staining than citrate or heparin anticoagulant. Vacutainer tubes containing EDTA anticoagulated blood can be submitted to the State Laboratory for analysis.

The time of specimen collection is important with malaria, but less important in other filarial infections. Malaria parasites are most numerous about midway between chills. One specimen taken at this time and a second specimen collected 5 - 6 hours later is ideal. Because of nocturnal periodicity in filarial infections, the specimen should be taken between 10 PM and 2 AM. In *Loa loa*, there is diurnal periodicity, and these specimens should be collected between 10 AM and 2 PM.

Ectoparasites

Ectoparasites are typically wingless arthropods. These include ticks, mites, fleas, lice, etc. Specimens of ectoparasites should be preserved and shipped in 70% ethanol.

Shipment of Specimens

Fill out the *Microbiology Submission Form #2*. Include the patient's name, date of birth, address, submitting agency, test request, and other pertinent information on the form. Identify the specimen with the patient's name and date of collection. **Make sure that identification on the specimen matches the form**.

Specimens sent through the mail must be in containers that meet postal regulations for infectious materials. Specimen containers should be placed inside a double mailing container, which consists of an inner metal case with a screw cap placed within a screw-capped outer cardboard container.

Mailed stool specimens require use of a preservative, and a two-vial method of collection and shipping is advocated. One vial contains 10% formalin, and the other contains PVA fixative. Thus the laboratory has a formalized specimen that can be examined for helminth eggs and cysts, and the PVA specimen can be examined for trophozoites and to a lesser degree, for cysts.

See Section 10: Sample Submission Guidelines.

Reporting and Interpretation of Results

Specimens received for parasitology will be processed daily. Stools for intestinal parasites will be concentrated and observed microscopically for distinct characteristic morphology. Results will be reported by laboratory-computerized report to the submitting agency. Blood smears will be examined for blood parasites, and forwarded on to the CDC for confirmation of results. A preliminary report will be generated by the State Laboratory indicating that the specimen has been forwarded on to the CDC. The final report will be generated upon issuance of a report from the CDC. Ectoparasites will be forwarded on to the Vector-Borne and Zoonotic Disease Section (VBZD) of the Bureau of Epidemiology and Disease Control for identification. The VBZD will issue a report directly to the submitting agency.

Section 5: Serology

The Serology Section is responsible for performing diagnostic testing for communicable diseases in support of outbreak investigations, and reference testing for private and public laboratories. The time required to process a microbiology specimen varies considerably, as indicated by the following table. Detailed information on the collection and submission of laboratory samples for any of the following tests can be obtained in the narrative guidelines that follow.

Agent	Specimen Required	Test Method ¹	Reference Values ²	Turn Around Time (TAT)
Adenovirus	Acute and convalescent sera	CF	<1:8	3 days
Brucella³	Single or paired sera	ТА	<1:20	3 days
Coccidioides immitis	Serum, CSF, Body Fluid	CF IDCF IDTP	<1:2 Negative Negative	3 days
Chlamydia	Acute and convalescent sera	CF	<1:8	3 days
CMV ⁴	Single serum	CF	<1:8	3 days
CNS Panel: East Eq. Encephalitis West Eq. Encephalitis St. Louis Encephalitis Venezuelan Encephal	Acute and convalescent sera	CF	<1:8	3 days
Colorado Tick Fever	Acute and convalescent sera	CF	<1:8	3 days
Dengue ⁵	Acute and convalescent sera	CF	<1:8	3 days
Hantavirus ⁶ : IgG	Serum	EIA (IgG)	Negative	1 - 2 days
IgM	Serum	EIA (IgM)	Negative	1 2 days

Agent	Specimen Required	Test Method ¹	Reference Values ²	Turn Around Time (TAT)
Hepatitis Diagnostic Panel: HbsAg	Serum	EIA	Negative	
HbcIgM	Serum	EIA	Negative	4 days
HAV IgM	Serum	EIA	Negative	
Hepatitis A Total Ab ⁷	Serum	EIA	Negative	7 days
Hepatitis B Prevaccine ⁷ : HBcIgG	Serum	EIA	Negative	7 days
Hepatitis B Postvaccine ⁷ : HBsAb	Serum	EIA	Positive	7 days
Herpes Simplex	Acute and convalescent sera	CF	<1:8	3 days
Histoplasma: Yeast phase	Acute and convalescent sera	CF & ID	<1:8	3 days
Mycelial Phase	Acute and convalescent sera	CF & ID	<1:8	3 days
HIV ⁸ : Screen	Serum/Oral fluid	EIA	Nonreactive	2 days
Confirmation	Serum/Oral fluid	WB	Negative	4 days
Influenza A	Acute and convalescent sera	CF	<1:8	3 days
Influenza B	Acute and convalescent sera	CF	<1:8	3 days
LCM	Acute and convalescent sera	CF	<1:8	3 days
Lyme ⁹	Serum	EIA	Negative	7 days
Measles Immune Screen	Serum	IFA	Positive	7 days

Agent	Specimen Required	Test Method ¹	Reference Values ²	Turn Around Time (TAT)
Measles Diagnostic: IgG	Acute and convalescent sera	CF	<1:8	3 days
IgM^{10}	Single Serum	EIA	Negative	2 days
Mumps Diagnostic: IgG	Acute and convalescent sera	CF	<1:8	3 days
${ m IgM^{10}}$	Single serum	IFA	Negative	2 days
M. pneumoniae	Acute and convalescent sera	CF	<1:8	3 days
Parainfluenza 1	Acute and convalescent sera	CF	<1:8	3 days
Parainfluenza 2	Acute and convalescent sera	CF	<1:8	3 days
Parainfluenza 3	Acute and convalescent sera	CF	<1:8	3 days
Respiratory Virus Panel: Influenza A Influenza B Parainfluenza 1 Parainfluenza 2 Parainfluenza 3 M. pneumoniae Adenovirus RSV Chlamydia	Acute and convalescent sera	CF	<1:8	3 days
Rickettsial Panel: Spotted Fever Group Typhus Fever Group Q Fever	Acute and convalescent sera	IFA	<1:16	7 days
RSV	Acute and convalescent sera	CF	<1:8	3 days
Rubella Immune Screen	Serum	EIA	Positive	2 days

Agent	Specimen Required	Test Method ¹	Reference Values ²	Turn Around Time (TAT)
Rubella Diagnostic: IgG	Acute and convalescent sera	EIA	EIA Negative	
IgM^{10}	Single serum	EIA	Negative	2 days
SARS	Acute and convalescent sera	EIA		2 days
Syphilis ¹¹ : Screen	Serum, CSF	VDRL	Nonreactive	2 days
Confirmation	Serum	FTA	Nonreactive	5 days
Toxoplasma	Serum	EIA	Negative	7 days
TORCH Panel: Toxoplasma Rubella CMV Herpes	Serum Serum Serum Serum	EIA EIA CF CF	Negative Negative <1:8 <1:8	7 days
VZ Immune Screen	Serum	IFA	Positive	7 days
VZ Diagnostic	Acute and convalescent sera	CF	<1:8	3 days
West Nile	Serum	EIA		

1. Test abbreviations

CF - Complement Fixation

EIA - Enzyme Immunoassay

FTA - Fluorescent Treponemal Antibody

IDCF - Immunodiffusion for CF antibodies

IDTP - Immunodiffusion for Tube Precipitin antibodies

ID - Immunodiffusion

IFA - Indirect Fluorescent Antibody

TA - Tube Agglutination

VDRL - Venereal Disease Research Laboratory test

- 2. A four-fold rise in antibody titer between acute and convalescent serum samples is indicative of a seroconversion, indicating evidence of recent exposure to the microbial agent.
- 3. A single titer of 1:160 by tube agglutination is considered diagnostic for Brucellosis.

- 4. When a markedly elevated CF antibody is detected, a congenital defect is detected suggestive of CMV, or illness accompanying immune suppression, it is highly recommended that attempts be made to isolate the CMV Virus.
- 5. Significant cross-reactions may be seen within the viruses in the Flavivirus group, including Dengue and St Louis Encephalitis (SLE).
- 6. Specimens submitted for Hantavirus testing are run for both IgG and IgM antibodies. Demonstration of the presence of IgM antibody is suggestive of recent exposure to Hantavirus (Sin Nombre Virus).
- Hepatitis A & B immune status testing is provided on a limited basis to the county health departments. Large scale screening of populations require prior approval from the Arizona Department of Health Services.
- 8. Samples submitted for HIV testing are screened by the Enzyme Immuno Assay (EIA). Samples that test positive by EIA are retested in duplicate. Those that repeatedly test reactive by EIA are subjected to a Western Blot confirmation test. This testing algorithm follows the guidelines established by ASTPHLD and the CDC.
- 9. Lyme Disease is not endemic to the state of Arizona. Therefore, all requests for Lyme Disease must be approved by the Vector and Zoonotic Disease Section of the Bureau of Epidemiology and Disease Control.
- 10. Testing is available for IgM antibody to Measles Mumps, and Rubella. However, due to the variability in the presence of IgM antibody in individuals, it is highly recommended that a convalescent serum specimen be collected if the IgM antibody test is negative and the clinical symptoms suggest the viral infection. IgM antibody may be absent if the specimen was collected too early in the course of infection, too late in the course of infection or in the instance of disease due to vaccine failure.
- 11. Serum samples submitted for antibody testing to Syphilis are screened by the VDRL, which is a non-treponemal test. Non-treponemal tests can be used for initial screening and for observing the patient's response to treatment. A non-reactive test may be interpreted as no current infection or an effectively treated infection.

Samples that test reactive by the VDRL are subjected to a confirmation test, by FTA. Use of this treponemal test should be reserved for confirming reactive non-treponemal tests, and for assisting in the diagnosis of late syphilis. Treponemal test misinterpretation often results from misuse of the treponemal test as a screening procedure. About 1% of the general population has false-positive results with the treponemal tests.

VDRL is the only standard serological test for Syphilis from spinal fluid. A reactive VDRL test on CSF usually indicates past or present infection of the central nervous system.

Specimen Collection

For serological tests, 10 to 15 ml of whole blood should be collected aseptically in a red top vacutainer tube. For pediatric patients, smaller volumes of blood may be collected in pediatric tubes. After collection, the red top tube may be transported directly to the State Laboratory or the tube may be centrifuged and the serum poured off into a separate vial. The optimal volume of serum for routine submissions is 2 - 3 ml.

Many tests require both acute and convalescent serum samples to be run in parallel on the same test run looking for a rise in antibody titer. A four-fold rise in antibody titer between the acute and convalescent samples is indicative of a sero-conversion, indicating evidence of recent exposure to the microbial agent. The acute sample should be drawn as soon as possible after appearance of symptoms. The convalescent sample should be drawn 10 - 14 days after the acute sample.

Other specimens that may be sent to the State Laboratory for serological testing include cerebrospinal fluid (CSF), pleural fluid, peritoneal fluid, and joint fluid. Approximately 2 ml of sample is requested for testing. However, since these samples are difficult to obtain, all attempts will be made to test the samples if less than the ideal sample amount is submitted. Store samples refrigerated and do not freeze. Submit on cool packs or wet ice.

Samples may be considered unacceptable if they are grossly hemolyzed, contaminated with bacteria, lipemic, leak in transit, or are improperly labeled. Samples must be transported with the appropriate paperwork, verifying that the information appearing on the specimen matches that on the submission form. Since the integrity of the sample must be maintained from the time of collection of the sample until testing is completed, labeling errors will result in rejection of the specimen.

Laboratory submission forms should be filled out completely with all pertinent demographic information. Successful tracking of positive cases is reliant on complete and accurate information being supplied on these forms, including patient name or identifier, date collected, date of onset of illness, submitter's name and address, and agency code.

For HIV serological testing, specimens are to be submitted with an HIV Submission Form. All other serological specimens should be accompanied with a Microbiology Submission Form #1 (Serology).

Shipment of Specimens

The specimen should be transported to the State Laboratory as soon as possible. Due to the intense heat seen in the Phoenix area in the summertime, it is advisable to ship the specimens cold to prevent damage to the specimen in transit, or overgrowth with bacteria. Whole blood samples may be sent on cool packs, but should never be frozen. Freezing whole blood will cause lysis of the blood cells, and render the blood sample unsatisfactory for testing. Serum samples, if not tested within 7 days, should be stored frozen and shipped to the State Laboratory on ice. Specimens may be mailed or delivered by courier to the State Laboratory.

See Section 10: Sample Submission Guidelines.

If Sent by Courier

- Blood and blood products sent in vacutainer tubes should first be placed in a plastic falcon tube to reduce the risk of shattering while in transit.
- The specimen should then be placed in a plastic specimen bag with separate compartments for the submission form and specimen.
- Pack the specimen and its form in absorbent material to help prevent breakage.

Note:

It is still acceptable to send more than one specimen together, as long as they are properly secured and identified. Please see example on the following page.

If Sent by Mail

- Blood and blood products sent in vacutainer tubes should first be placed in a plastic falcon tube to reduce the risk of shattering while in transit.
- Wrap the submission form around the falcon tube, and place the falcon tube inside a styro-foam container or cardboard mailer. Pack the specimen and its form in absorbent material to help prevent breakage

Note: Do not put the submittal form inside the falcon tube or wrap the specimen inside the submittal form. This is very unsafe.

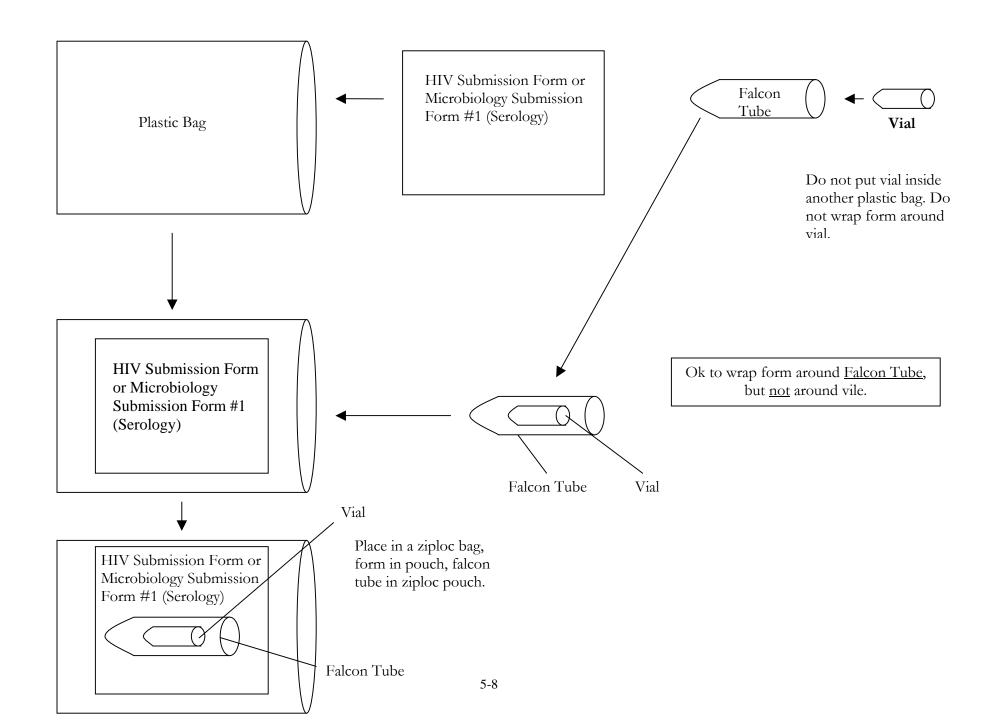
• Place appropriate biohazard label on the outside of the mailing container before transportation to the State Laboratory.

Falcon tubes and cardboard mailers are available from the State Health Laboratory Receiving Section, (602) 542-1190. Please call your orders in advance to insure prompt service and delivery.

Specimen Rejection Criteria

- Specimen not properly identified
- Identification on specimen does not match submittal form
- Broken in transit
- Leaked in transit
- Grossly hemolyzed, lipemic, turbid, or grossly contaminated
- No convalescent serum received

The submitter will be notified of all rejected laboratory specimens by telephone and with a laboratory report mailed to the submitting agency confirming the reason for rejection.



Section 6: Virology

The following table briefly outlines the viral culture services offered at the State Laboratory. The time required to process specimens and to render a final report may vary considerably depending upon the nature of the clinical material, the type of virus isolated, and whether or not any virus is isolated. The turnaround times listed in the table are the expected turnaround times to report a negative culture.

Organism/Disease	Specimen	Transport Medium	Comments	Turn Around Time (TAT)
Adenovirus	Throat, N/P, Eye	Hanks		14 days
Arbovirus	Throat, N/P, CSF, Tissue (autopsy)	Hanks	Contact Virology Lab before specimen submission	14 days
Arbovirus (Mosquito surveillance)	Mosquito pools	None	Transport frozen	14 days
Coxsackie A Virus	Stool, N/P, Throat, CSF	Hanks		14 days
Coxsackie B Virus	Stool, Throat, N/P, CSF, Pericardial Fluid	Hanks		14 days
Cytomegalovirus (CMV)	Urine, Throat, N/P, Bronch Wash, Biopsy, Whole Blood	Hanks	Urine should be transported within 24 hours (store at 4° C)	4 weeks
Echovirus	Stool, Throat, N/P, CSF	Hanks		14 days
Enterovirus	Stool, Throat, N/P, CSF	Hanks		14 days
Herpes	Lesions, Vesicles, Throat, N/P, Rectal	Hanks	Typing is not conducted	7 days
Influenza	Throat, N/P	Hanks	Do not freeze	14 days
Measles (Rubeola)	Throat, N/P, Urine, Whole Blood	Hanks		14 days
Mumps	Throat, N/P, Sputum, Urine, CSF	Hanks		14 days
Parainfluenza	Throat, N/P, Sputum	Hanks		14 days

Organism/Disease	Specimen	Transport Medium	Comments	Turn Around Time (TAT)
Polio	Stool, Throat, N/P, CSF	Hanks		14 days
Rhinovirus	Throat, N/P	Hanks		14 days
RSV	Throat, N/P	Hanks		14 days
SARS	Sputum, O/P, N/P	None		
Varicella-Zoster	Vesicle Fluid	Hanks		21 days
West Nile				

Collection

In order to optimize the ability of the Virology Section to isolate and identify viral agents from clinical specimens, it is very important that the specimens be collected, handled, and transported in a manner that minimizes deleterious effects on any viral agents present. In addition, sufficient information should be provided with a submitted specimen to guide the laboratory in the selection of proper inoculation techniques for the viral agents suspected.

Nasopharyngeal/Throat

Virus isolation is most successful if respiratory specimens are collected within 3 to 5 days of onset of illness. Swabs from both the throat and nasal passage should be collected. The pharynx is swabbed vigorously with a cotton swab moistened with collection medium free of serum such as Hanks, and then placed in a transport container containing Hanks Buffered Saline Solution (HBSS). Break off the ends of the applicator sticks leaving the swab tips in the collection medium. Calcium alginate swabs are unacceptable for submission of specimens for viral culture. Specimens submitted for Influenza should not be frozen.

NP swabs are used to collect specimens from the nasal passage. Allow the swabs to remain in the nasal passages for a few seconds to absorb the nasal secretions laden with virus. Place the swabs in the Hanks BSS and label vial.

Store specimens frozen at -70° C if they cannot be inoculated within 48 hours. Transport to the laboratory on wet ice.

Rectal

Collect the specimen no later than 7-10 days after onset of illness. Use a cotton or Dacron tipped swab moistened with Hanks BSS solution to insert 4 - 6 cm into the rectum. Rub the mucosa until visible fecal material is present. Two swabs should be collected in this manner. Place the swabs into Hanks BSS and break the ends of the swabs. Store frozen at -70° C if specimens cannot be transported to the laboratory within 48 hours.

Urine

Urine specimens are generally tested for Cytomegalovirus, although Measles and Adenovirus can sometimes be found in urine. Collect the specimen as soon as possible after onset of illness. Clean voided specimens (10 - 20 ml) are collected in sterile containers and transported immediately to the laboratory on wet ice or cool packs. If urine is to be cultured for CMV, it must be transported to the laboratory as soon as possible, preferably within 24 hours. Specimens should be stored at 4°C and transported on wet ice or cool packs. DO NOT FREEZE URINE FOR CMV.

Note: Specimens collected for both RSV and Influenza can only be frozen at < -70°C.

Throat Washings

Throat washings should be collected by gargling with HBSS. DO NOT FREEZE SPECIMENS COLLECTED FOR ISOLATION OF RSV.

Cerebrospinal Fluid (CSF)

For virus isolation, 3 - 4 ml of CSF should be collected no later than 7 - 10 days after onset of illness. Place in a sterile screw capped tube <u>without</u> collection medium. If delays in transport, store frozen at -70° C. Transport to the laboratory on wet ice or cool pack.

Cervical

Specimens should be collected using a speculum. A swab is used to clear the cervix of mucus, and is then discarded. A second swab is then inserted into the cervical canal (approximately 1 cm) rotated, and left in place for a few seconds to absorb secretions. If lesions are seen, these should be swabbed. Swabs are placed in a transport tube containing Hanks and transported to the laboratory on wet ice or cool packs.

Eye Specimens

A swab moistened with sterile saline is used to collect secretions from the conjunctiva. Place the swab in the viral collection medium (Hanks).

Scrapings from the cornea or conjunctiva should be collected by an ophthalmologist or trained physician and placed in Hanks Solution.

Stools

Place three to four grams of stool into a sterile container and transport to the laboratory on wet ice or a cool pack.

Vesicular Lesions

Vesicular fluids and cellular material from the base of lesions should be collected for virus isolation during the first three days of the eruption. The fluids should be diluted in 2 - 3 ml

of Hanks virus collection medium to prevent clotting. Alternatively, the fluids may be collected on a swab and then placed into Hanks solution. Store refrigerated for up to 48 hours. If specimens are to be held for longer than 48 hours, store frozen at -70° C. Transport to the laboratory on wet ice or cool packs.

Blood

Although blood is not the optimal specimen for isolation of most viruses, it may be used for the recovery of some of the vector-borne viruses, enteroviruses, and CMV. Specimens for virus isolation should be collected as soon as a viral agent is suspected, otherwise early neutralizing antibody may prevent isolation of virus from the blood. Either serum or leukocyte preparations may be used for viral isolation. For isolation of virus from leukocytes, 8 ml of blood is collected in a tube containing an anticoagulant, preferably EDTA (heparin has been shown to inactivate Herpes virus). For isolation of virus from the serum or blood clot, 8 ml of blood is collected aseptically without an anticoagulant. Transport on wet ice or a cool pack.

Autopsy or Biopsy Specimens

Autopsy specimens should be collected within 24 hours after death. Samples from probable sites of pathology are collected using separate, sterile instruments and separate sterile containers for each specimen. Tissues are transported to the laboratory on wet ice or cool pack. If they cannot be tested within 48 hours, they should be stored frozen at -70° C.

Shipment of Specimens

Place specimens in plastic baggy or aluminum can with secure cap. Place in a Styrofoam shipping container with adequate ice or cool packs. Each specimen must be accompanied with a *Microbiology Submission Form #2*. Mail, ship or courier specimens to the State Laboratory.

See Section 10: Sample Submission Guidelines.

Reporting and Interpretation of Results

Specimens are read daily for typical cytopathic effect (CPE). Turnaround time for negative cultures varies from one to five weeks depending upon the viral syndrome suspected. Genital Herpes cultures are held for 7 days before reporting as negative. Respiratory and enteric virus cultures are held for 2 weeks. CMV cultures are held for 4 weeks. Delays in reporting may be due to cultures that have one to several passages. In addition, cultures yielding virus isolates may require more or less time for identification of the virus, depending upon the isolate involved. Failure to isolate a virus should not rule out a virus as a cause of clinical illness.

Rabies

Collection

The head of animals the size of dogs or smaller should be submitted. The head should be severed close to the shoulders allowing sufficient tissue of the throat to remain, to insure inclusion of salivary glands.

The brain of large animals, such as cows and horses, should be removed by a veterinarian and sent to the laboratory unless prior arrangements have been made.

Small animals such as bats, mice, rats, and gophers may be sent intact.

Please Note: Rodents will be tested only by prior approval from the Vector-Borne and Zoonotic Disease Section of the Bureau of Epidemiology and Disease Control. Contact them at 602-364-4562 for instructions. Rodents may carry other serious and deadly diseases, such as Plague, Tularemia, or Hantavirus, and should be handled with extreme caution.

Birds and reptiles will not be accepted for examination.

Specimens for rabies examination should be collected immediately after the death of the animal. Decomposed specimens or specimens infested with maggots cannot be tested. Exceptions to this situation will be evaluated on a case-by-case basis. If unsure, submit the sample and laboratory staff will evaluate the condition of the animal.

Shipment of Specimens

Specimens for rabies should be submitted in a double plastic bag. Place the bag in a Styrofoam shipper filled with wet ice or cool packs. Complete a Rabies Submission Form and place the form inside the shipping container along with the specimen, but <u>outside</u> the plastic bag. Ship the specimens to either the Phoenix or Tucson State Laboratory. Testing delays may be experienced on specimens that are received frozen.

See Section 10: Sample Submission Guidelines.

Reporting and Interpretation of Results

In all cases when exposure of a human is reported by a physician or veterinarian, laboratory examination will be made consisting of microscopic examination of smears prepared from brain material. The results of the microscopic examinations will be available 24 to 48 hours after receipt of the specimen. Positive results will be reported by telephone to the submitting agency and to the Vector-Borne and Zoonotic Disease Section of the Bureau of Epidemiology and Disease Control.

Section 7: Newborn Screening

For many types of genetic or metabolic diseases, early diagnosis and treatment is critical. Although babies born with these disorders may appear to be normal at birth, with time these disorders may have a devastating effect on the infant's health and development. In most cases, early screening, detection, and treatment of these disorders can result in normal growth and development.

The Arizona State Laboratory has been awarded the contract as the Central Screening Laboratory to provide testing services for the Arizona Newborn Screening Program. The State Laboratory receives all newborn screening specimens in Arizona and conducts initial testing for seven endocrine/metabolic disorders plus hemoglobinopathies (including Sickle Cell Anemia), and provides confirmatory testing for the abnormal hemoglobins. The seven endocrine/metabolic disorders screened include the following:

- Congenital Adrenal Hyperplasia (CAH)
- Congenital Hypothyroidism
- Phenylketonuria (PKU)
- Galactosemia
- Homocystinuria
- Maple Syrup Urine Disease
- Biotinidase Deficiency.

For most of these disorders, the incidence in the population is low, but the potential for devastating consequences and the high cost of treating infants who possess the disorders is thought to justify the cost of mass screening.

Specimen Collection

The State Laboratory has developed two-specimen collection kits listed below:

Linked Kit

Use of the Linked Kit allows for linkage of the first and second Newborn Screen Specimens. Names of newborn infants frequently change during the first weeks of life. The linked kits will allow better identification of infants. These paired kits have a common collection kit number, which is used to link first and second specimens on the same babies. The first specimen kit is used to collect the heel-stick specimen at the hospital or birthing institution prior to the baby's discharge. The second specimen of the linked kit is sent home with the mother for use by the baby's Primary Care physician at the first well-care visit.

Supplemental Kits

The Supplemental Kit is used in institutions when the linked kit is lost or otherwise unavailable, to collect a sample for a repeat test to follow-up a previously tested positive result, or for the repeat of an unsatisfactory specimen.

Regardless of which specimen collection kit is used, <u>all babies</u> born in Arizona are required to have a Newborn Screen performed. It is the responsibility of the birthing institution to assure that a Newborn Screen is collected within 72 hours or prior to discharge. The state of Arizona also mandates the collection of <u>second screen specimens for all babies born in Arizona.</u> A second screen is also required when the first screen specimen is collected at less than 24 hours of age. The second screen specimen should be collected between 7 and 14 days.

The following outlines the procedure for Heel Stick specimen collection:

- 1. Warm the infant's foot for approximately 3 minutes with a warm, moist towel or foot warmer (heated to a temperature no higher than 42° C) to increase the blood flow. Hold the foot in a position, which increases venous pressure (lower than the heart so that blood will pool in the heel).
- 2. Disinfect the skin with an alcohol pad (70 % isopropanol) and dry with sterile gauze, sterile cotton ball, or air dry.
- 3. Puncture the skin on the heel using a sterile lancet or automated lancet device with a tip no longer than 2.4 mm. A longer point could pierce the heel bone. Use the most medial or lateral portion of the plantar surface of the heel. Do not use previous puncture sites or the curvature of the heel. Do not perform skin punctures on the central area of the infant's foot. This may result in injury to the nerves, tendons or cartilage. Wipe away and discard the first drop of blood (using sterile gauze), since it may be contaminated with disinfectant or tissue fluids. Note: In small premature infants the heel bone may be no more than 2.0 mm beneath the plantar heel skin surface. Puncturing deeper than 2.0 mm may be excessive and a lancet of length 1.75 mm or less is recommended to prevent bone damage.
- 4. Allow the second drop of blood to form by spontaneous free flow of blood. CAUTION: Milking or squeezing the heel at the site of the puncture may cause hemolysis of the specimen or a mixture of tissue fluids with the specimen. This would be cause for rejection of the Newborn Screening specimen.
- 5. Touch the drop of blood to the center of the first filter paper circle. The paper must not be pressed against the puncture site on the heel. Fill the circle with the single application of the filter paper to the heel. Apply blood to one side of the filter paper only. After filling the one circle, proceed with filling the remaining circles. It is important to make sure that the circles are completely filled. Both sides of the filter paper should be examined to assure the blood uniformly penetrated and saturated the paper.
- 6. Air-dry the filter paper at room temperature (15° C to 22° C) in a horizontal position away from direct sunlight for at least 3 hours.

Shipment of Specimens

Assure that all patient demographic information has been filled out completely on the laboratory submission form before specimen collection. Specimens must be completely dry before inserting in a mailing envelope. Do not package the dried blood spot specimens inside a sealed plastic bag. The lack of air exchange inside of a sealed plastic bag may cause heat buildup, moisture accumulation, and/or chemical leaks from the plastic, which can damage specimen integrity. Within 24 hours of collection, the specimens should either be mailed or sent by courier to the Arizona State Laboratory in Phoenix. The State Laboratory in Phoenix should receive specimens within 3 - 4 days of collection to allow for rapid detection of these serious disorders. Specimens not received within 14 days of collection will be rejected as "unsatisfactory, too long in transit".

See Section 10: Sample Submission Guidelines.

Unacceptable Specimens

Specimens are rejected as unsatisfactory to test for the following reasons:

- Inadequate blood collection (QNS) The specimen is considered QNS if there is insufficient specimen to punch the blood spots for the laboratory tests.
- Contamination Gross contamination with alcohol, water, or other foreign substance.
- Tissue Fluids Caused from squeezing or milking the puncture site during specimen collection.
- Layered Application of successive drops of blood to the same printed circle.
- Scratched or Torn A result of improper application of the sample through use of capillary tubes.
- Too long in Transit Specimens must be received within 14 days of collection.
- No Blood No collection was made.
- No identification Specimen was not properly identified to assure the integrity of the specimen.

Reporting and Interpretation of Results

Normal laboratory results are reported as "Normal". Abnormal results are reported as "Abnormal", with quantitation of test values provided when applicable. All abnormal reports are sent to the Newborn Screening Program, which is responsible for tracking and case management of positive cases. Results that are determined to require emergency notification are phoned directly to the Arizona Newborn Screening Follow-up Program within the hour. Laboratory mailers are generated

within 24 hours of completion of laboratory testing and are mailed to the submitting agency and the physician of record.

NORMAL RANGES:

17 – OHP (CAH) <50 ng/ml*
Phenylalanine <2.1 mg/dl
Methionine <2.0 mg/dl
Leucine <4.0 mg/dl

Galactose >2.4 GALT Units/gHb

T4 >6.0 ug/dl
TSH <20.0 uIU/ml
Biotinidase Enzyme Present

Hemoglobin FA

*Birth weight adjustable

Section 8: Environmental Microbiology

The Environmental Microbiology Section conducts microbiological examinations of food and water for sanitary quality and isolation and identification of microorganisms of public health significance. Sanitarians and representatives of federal, state, county, and city agencies responsible for monitoring quality and enforcing regulations governing production and handling of food and water, may submit samples for analysis.

Food Product Samples

In order to ensure rapid and efficient service, communication with the Environmental Microbiology Section is very important. Before submitting or shipping any samples for analysis, please call the State Laboratory.

A three-day food history and investigation observation should be used to guide the selection of appropriate foods for analysis. An investigation should be conducted before submitting samples to the lab for analysis. The following information must be provided with the samples at the time of submission:

- Symptoms
- Incubation period
- Duration of illness
- Physician's diagnosis, and
- Results of any clinical tests or cultures

Collection

After determining the appropriate food specimen to submit, aseptically collect approximately 200 grams of a solid product or about 100 ml of a liquid. Collection should be in a sterile whirl-pak plastic bag or sterile urine collection cup. The State Laboratory does not provide sterile collection containers for food collection.

Shipment of Specimens

All samples must be kept cold (<10°C) during transit to the laboratory. Samples that are shipped should be placed in a leak-proof shipping container, preferably a Styrofoam container, packed with sealed cold packs (i.e. blue ice packs). Samples that are hand delivered on wet ice should be protected from cross contamination as the ice melts during transit.

See Section 10: Sample Submission Guidelines.

A properly completed *Bacterial Food Analysis Submittal*/Report Form must accompany **each individual sample**. Each sample must be identified by a number that corresponds to the same identification number written in the submitter sample information on the submission form. More detailed information regarding how to obtain collection/submission supplies can be found in Section 11: Requesting Collection Kits and Mailing Containers.

Reporting and Interpretation of Results

Quality control samples are tested for aerobic plate count, total coliforms, fecal coliforms and *E. voli*. Pathogen isolation and identification is available for foods implicated in food borne illness outbreaks. Tests available include, but are not limited to, the following:

- Staphylococcus aureus plate count
- Bacillus cereus plate count
- Clostridium perfringens plate count
- Yeast and mold count
- Salmonella isolation
- *Campylobacter* isolation
- Listeria isolation
- E. coli 0157:H7 isolation
- Filth analysis
- Foreign object identifications
- Container analysis

Food samples are analyzed according to methods specified in the Bacteriological Analytical Manual (FDA BAM) or by methods specified by the National Centers for Disease Control (CDC). When appropriate, rapid analytical test kits are used to screen samples for pathogens to provide quicker test results during food outbreak investigations or emergencies. The rapid test results usually take only 1 to 2 days. However, positive results of these tests are only presumptive and conventional tests need to be done to confirm these results.

Preliminary results are usually available within 48 to 72 hours after processing has begun. Confirmatory test results are usually available within 48 hours to ten days depending on the test organism. Please contact the Environmental Microbiology Section at (602) 542-6130 at any time for updates on the progress of the testing. Generally, final reports are mailed out 3 to 11 days after initial processing begins.

Interpretation of lab results is the responsibility of the submitter. The laboratory will consult with the submitter, if requested. No legal food standards are available on most products, so care and common sense are needed in the interpretation of lab data. Use your experience and comparisons to evaluate the results.

Water Samples

The laboratory tests drinking water for the presence/absence of coliforms and *E. voli* in compliance with the Safe Drinking Water Act. In addition, the laboratory tests surface or source waters, wastewater and runoff waters for indicator organisms and occasionally pathogens. Please call the State Laboratory before submitting or shipping water samples for analysis. However, it is not necessary to call the laboratory before submitting <u>routine</u> drinking water samples.

Collection

Drinking Water Samples

Drinking water samples should be collected in sterile four-ounce whirl-pak bags or sterile collection bottles with sodium thiosulfate added to neutralize any chlorine in the water. Aseptically collect about 125 ml of water from the sample tap. If using the whirl-pak bags, be sure to whirl them closed tightly and tie the tabs together securely.

Other Water Samples

Surface water, source waters, runoff waters, etc. can be aseptically collected in any appropriate size sterile whirl-pak bag or bottle (sodium thiosulfate is not needed); however, at least 125 ml is needed to test.

Shipment of Specimens

Drinking Water Samples

Drinking water samples <u>must be received and tested within 30 hours of collection</u>. Samples may be mailed or sent by courier to the State Laboratory to arrive the next day. Drinking water samples do not need to be iced during transit. Each sample must be accompanied by a properly completed *Drinking Water Microbiological Analysis Submittal/Report Form*. For compliance samples, the submitter must complete all of the red areas on the left of the form or the sample may be rejected. Information regarding how to obtain collection/submission supplies can be found in Section 11: Requesting Collection Kits and Mailing Containers.

Other Water Samples

These waters need to be received in the laboratory within six hours of collection, and must be iced during transit. Since the transit time is so short, it is usually best to send the water samples to the laboratory by courier. A properly completed *Water Microbiological Analysis Submittal/Report Form* must accompany each sample. More detailed information regarding how to obtain collection/submission supplies can be found in Section 11: Requesting Collection Kits and Mailing Containers. Before submitting these water samples, please call the Environmental Microbiology Section at (602) 542-6130 to arrange for testing.

See Section 10: Sample Submission Guidelines.

Reporting and Interpretation of Results

Drinking Water Samples

Drinking water samples are routinely tested for the presence of total coliforms and *E. coli* using the enzyme substrate coliform test. This method provides results in 18 to 24 hours. The Standard Methods PA (Presence-Absence) coliform test is occasionally used, which provides results in 48 to 96 hours. These are both EPA approved methods.

Results of drinking water coliform tests are usually available within 18 to 24 hours after processing has begun. All positive results are called to the submitter, providing that a telephone number has been supplied. In addition, all compliance positive results and repeat samples are faxed to ADEQ. Leaked in transit and too long in transit samples are also called to the submitter. Final reports will usually be mailed one to two days after initial processing. If the sample is checked as a compliance sample, a copy is sent to the submitter and ADEQ.

Normally, the maximum contaminant level for total coliforms in drinking water is based on the presence or absence of coliform organisms in a 100 ml sample. A single water sample can have 0 coliforms per 100 ml. Other rules apply when more routine samples are collected, as the ADEQ compliance Department dictates. The number of samples required is based on the population served by a public water system. If a compliance sample is positive, repeat samples need to be collected. Please contact your ADEQ compliance officer to determine the number and location to collect these repeat samples.

Other Water Samples

Other types of waters are tested for indicator organisms such as fecal coliforms, *E. voli*, fecal strep and enterococcus using either a Most Probable Number (MPN) method or a Membrane Filter (MF) method. The methods are Standard Methods. (A list of methods is outlined in the table below). On occasion, waters are tested for pathogens, such as *Salmonella*. Please contact the Environmental Microbiology Section for these requests.

Method		Standard	Holding	
Name	Units	Method Number	Time	Matrix
Presence/Absence	Presence or	SM 9221D	30	Drinking, Well or
(P-A) Coliform Test	Absence/100 ml	3111 92211)	Hours	Ground Water
Enzyme Substrate Coliform Test (Colilert/Colisure)	Presence or Absence/100 ml	SM 9223B	30 Hours	Drinking, Well or Ground Water
Colilert Most Probable Number (MPN) (Colitray)	MPN Index/ 100 ml	SM 9223B	30 Hours 6 Hours	Drinking, Well or Ground Water Surface/Ambient and Wastewater
Fecal Coliform Membrane Filter (MF)	C.F.U/100 ml	SM 9222D and SM 9221E	6 Hours	Surface/Ambient and Wastewater
Standard Fecal Coliform Fermentation (15 Tube M.P.N.)	MPN Index/ 100 ml	SM 9221B and SM 9221E	6 Hours	Surface/Ambient and Wastewater
E. coli Determination (E.C. with MUG)	C.F.U./100 ml or MPN Index/100 ml	SM 9221F	6 Hours	Surface/Ambient and Wastewater

Other waters and their testing results are usually available within 1 to 5 days, depending on the method used and the target organism. Call the Environmental Microbiology Section at (602) 542-6130 for an update at any time. Final reports are mailed to the submitter when all tests are completed. The significance of the results of other waters and their tests depends on the circumstances. Consult with the State Laboratory and ADEQ if needed.

Section 9: Epidemic Detection and Response (Bioterrorism)

Since the terrorist events of September 11, 2001, the Arizona State Laboratory has set guidelines for the submission of miscellaneous powders and other suspicious substances for detection of priority biological agents (i.e., anthrax, plague, etc.). Clinical specimens of patients exposed to an intentional release of priority biological agents are submitted according to the Bacteriology and Virology protocols stated in this manual. Environmental specimen submission is discussed below.

Please contact and alert the State Bureau of Epidemiology and Disease Control at (602) 364-3289 before submitting samples for potential outbreak or unusual suspect organisms. This is to include both patient and environmental specimens of a suspected intentional release of any biological agent. In the event that an intentional release of any biological agent is suggested, please be sure to also contact the local county health department, local law enforcement agencies, as well as the FBI field office at (602) 279-5511 to inform them of the incident.

Collection

State and local health department officials and persons with expertise in this area should be involved in the risk assessment and decision-making process. After determining if there is a legitimate threat or a suspicious substance, make sure that personnel do not directly handle, touch, smell, or otherwise closely inspect these samples. Also, limit the number of persons handling the specimen. Hazmat teams (typically the Fire Department or Sheriff's Office) should be involved in the handling, packaging and clean up of exposed areas.

Contain the evidence (a double-bagging with biological hazard bags would be appropriate). Also collect the names and contact information of those exposed to the said substance. "Exposed" can be defined as the individual opening the product and those within 6 feet of the product when it was opened.

For suspect bioterrorism specimens, please remember that **both** paperwork and the sample are considered criminal evidence and **will** be used in a court of law. A chain of custody must be maintained between whoever collects the specimens, and whoever subsequently handles the specimens until they reach and are directly accepted by the State Laboratory. It is best to minimize the number of people within this chain of custody, because all persons coming into possession of the specimens are subject to being called to testify in a court proceeding. To maintain the chain of custody, the specimens must be maintained within direct possession of the person responsible, or under lock and key, with all key holders becoming part of the chain of custody.

If someone is contaminated with a suspected substance, decontamination may be considered based on the extent of contamination, the amount of product involved, and the advice of public health officials. (Call the State Bureau of Epidemiology and Disease Control for further guidance at 602-364-3289). If someone simply opened a letter claiming to contain a biological agent but without obvious powders, full decontamination is probably not warranted. Health officials can evaluate the need for decontamination and the initiation of antibiotic prophylaxis. In almost all circumstances, the decision to initiate prophylaxis can be delayed until the presence or absence of a biological agent is determined.

Collected samples should be sent to the State Laboratory for testing within 7 days of the incident. Specimens involving human exposure should be immediately transported directly to the State Laboratory. Specimens should be of sufficient quantity and may consist of either water (drinking or surface), isolates for identification (submit both pass plate or slant **and** the original plate), soil, air samples, powders, or packages (sample requirements are outlined in the table below). Specimens submitted can be **no** larger than the following dimensions: 3'x14"x13". In cases of specimens larger than these dimensions, send in only the suspicious substance to be tested and store all other evidence according to local law enforcement protocols. No matter the package size, if powder is available in abundant supply, collect only a portion of the powder (up to 5 grams) and submit it to the State Laboratory. Seal and store the remainder of the package under custody until pathogen presence has been ruled out.

Sample Requirements

Specimen Type	Amount	Notes
Water ^a Drinking	100 mL – 1,000 mL	Collected aseptically ^a
Surface	250 mL – 1,000 mL	Collect two samples: aseptically ^a one at water surface, other at sediment layer by opening up sealed container next to sediment.
Soil	50g – 1000g	Collected 1" to 2" of surface soil.
Air	Up to 100L	Collected via collection device. Amount is dependent upon particle concentration. Refrigerate if specimen was collected into a bacterial growth medium.
Surface	Up to 100 mm ² (4 inch ²)	Use sterile swab or gauze. Synthetic fibers, synthetic or metal shafts strongly preferred.
Powder	Up to 5g	Collect aseptically.
Food ^a	5-100g	If food is not available, submit empty containers.
Isolate ^a	Isolate streak or slant Original isolation plate	Send in both plates or tubes.
Patient Specimen	See Bacteriology, Serology or Virology Sections.	

^a Refrigerate immediately and transport on ice. Keep good records and send the State Laboratory a copy.

Shipment of Specimens

Packaging and labeling protocols are the same for any infectious substance. However, it is best to remember that under federal law whomever packages the specimen for shipment is legally responsible for any nosocomial infections from the specimen or isolate that occur due to improper packaging from the time of packaging until the moment the specimen is delivered, unwrapped, inspected and determined to be intact. At that point the recipient takes legal responsibility for the specimen.

Please adhere to the following protocol when packaging any sample of a suspected priority biological agent for transport to the State Laboratory. (e.g. Packing Instruction 602)

- If the specimen is a dry powder or paper material, place it in a plastic zip-lock bag. A biohazard label should be placed on the outside of the zip-lock bag.
- Place the bag or specimen receptacle into a leak-proof container with a tight cover. The container should be labeled "biohazard".
- Place this container into a second leak-proof container with a tight cover. The container should be labeled "biohazard". The size of the second container should be no larger than a one-gallon paint can.
- Place the second container into a third leak-proof container with a tight cover. The container should be labeled "biohazard". The third container should be no larger than a five-gallon paint can.
- All containers should be properly labeled, and meet both state and federal regulations for transport of hazardous materials.

When transporting specimens to the State Laboratory, make sure that Laboratory personnel have been informed <u>prior</u> to the arrival of the specimen. Chain of custody must be maintained at all times whether sent via courier, UPS, Fed EX, USPS (*priority mail ONLY*), or other qualified commercial carrier according to State and Federal shipping regulations, as well as carrier requirements. Copies of all shipping documents must be retained by the submitter.

Reporting and Interpretation of Results

The results of environmental samples will be available 2-7 days after receipt of the specimen dependent upon the biological agent suspected. Results will be reported by the State Laboratory to the State Bureau of Epidemiology and Disease Control (602-364-3289) and this agency will contact the submitter and all other relevant agencies with the results.

Section 10: Sample Submission Guidelines

All diagnostic and infectious specimens must be transported to the Arizona State Laboratory according to regulations. If required, outer packages must be affixed with the proper labels, such as:

- Biohazard (OSHA)
- Diagnostic Specimen (IATA)
- Etiological Agent (CDC)
- Infectious Substance (DOT 6.2).

All samples and their containers must be identified with the appropriate labels and client information. Package specimens properly to protect them against breakage and leakage during transit. Any specimens which are leaking and/or not properly identified will be rejected. The following are brief guidelines for properly packaging specimens for submission.

- The inner packaging must comprise a watertight primary receptacle and a watertight secondary packaging. An absorbent material must be placed between the primary receptacle and the secondary packaging unless the specimen is a solid substance.
- The outside packaging must be of adequate strength. Some specimens must be kept cold
 during transportation and require either ice packs or dry ice. If ice packs are used, the outer
 package must be leak-proof. DO NOT place dry ice in hermetically sealed containers. If
 dry ice is used, the outer package must have the dry ice label (DOT 9) and also state the dry
 ice weight in kilograms.
- Place submitting forms on the outside of the primary receptacle. For submission of rabies specimens, place the submission form in an envelope and tape to the outside of the package.

The State of Arizona adheres to the Infectious Substances Shipping Guidelines (2nd Edition Issued January 2001) produced by the International Air Transport Association (www.iata.org/cargo/dg). All etiologic agent preparations and clinical specimens known or reasonably believed to contain an etiologic agent must conform to PHS/CDC's 42 CFR (72.3a), meet the packaging requirements of DOT's 49 CFR. Other requirements apply as outlined in DMM (C023.8.3 a-f).

Submit samples to any of the 3 locations below:

Arizona Department of Health Services Bureau of State Laboratory Services 250 North 17th Avenue Phoenix, Arizona 85007 (602) 542-1188 Tucson Regional Laboratory 416 West Congress Street Tucson, Arizona 85701 (520) 628-6360

Flagstaff Regional Laboratory 2500 Fort Valley Road PO Box 2800 Flagstaff, Arizona 86003 (928) 226-1154

Section 11: Requesting Collection Kits and Mailing Containers

Supplies ordered from the Arizona State Laboratory are to be used ONLY to submit specimens to the State Laboratory. There are two Request for Materials forms currently in use: a *Newborn Screening Supplies Request Form* and a *Request Form* for all other supplies available from the State Laboratory. Supplies from the Phoenix location can be requested by mailing, faxing or calling the Receiving Section at:

Arizona Department of Health Services Bureau of State Laboratory Services ATTN: Receiving Section 250 North 17th Avenue Phoenix, AZ 85007 Fax (602) 542-0760 Phone (602) 542-1190

Please request materials before they are required as the expected turn around time per order is **FIVE** business days. Most materials do have a limited shelf life; therefore, only order what will be used before the expiration date. Please do not use expired kits or any kits in which the medium has changed characteristics. Dispose of the media properly and order replacement supplies. The following table provides information regarding submission forms, kit contents and expiration period of each kit. Submitters may use the *Request for Materials Form* to order entire kits, as well as individual components.

KIT	CONTENTS	SHELF LIFE
Influenza Kit	Microbiology Submission Form #2 Instruction Sheet Dacron N/P Swab (metal handle) First Mailing Label Media: Hanks Blue Top. Store +2 to +8°C	2 months
Pertussis Kit	Microbiology Submission Form #2 Instruction Sheet Calgiswab Swab Mailing Label Media: Regan Lowe. Store +2 to +8°C	2 months
Ova & Parasite Kit	Instruction Sheet Baggie Metal Container Cardboard Mailer Media: PVA & Formalin. Store +20 to +25°C	6 months

KIT	CONTENTS	SHELF LIFE
Enteric Kit	Instruction Sheet Baggie Metal Container Cardboard Mailer Media: Cary Blair & Buffered Glycerol Saline (BGS). Store +20 to +25°C	6 months
Tuberculosis Kit	Sputum Vial Metal Container Cardboard Mailer Store +20 to +25°C	1 year
Bacti Water Kit	Instruction Sheet Drinking Water Microbiological Analysis Submittal/Report Form Mailing Label Whirl-pak bag Store +20 to +25°C	1 year
Chemistry sets	Nitric Acid, Non-Preserved, Sulfuric Acid Store +20 to +25°C	1 year
Chlamydia Kit: Male	50 male swabs per box Store +20 to +25°C	1-2 years
Chlamydia Kit: Female	50 female swabs per box Store +20 to +25°C	1-2 years